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ORIGINAL ARTICLES

REGULAR DEPARTMENTS

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A.M.A. ARCHIVES OF
PATHOLOGY

A New Experimental Method of Producing Acute Inflammation

BERNARD K. FORSCHER, Ph.D., and HAROLD R. STANLEY Jr., D.D.S., M.S., Bethesda, Md.

In order to study quantitatively the time sequence of chemical changes occurring in a specific tissue during an acute inflammatory response, it was necessary to have a simple technique for applying a standard reproducible insult to the experimental site. It was also required that no tissue be physically removed by the technique or that the integrity of the treated surface be destroyed at the time of stimulation, and that the lesion heal within two or three weeks. This report describes a new method for obtaining these results and reports some preliminary results obtained with its use. The changes following the application of this technique were studied histologically and chemically for qualitative and quantitative evaluation of the reproducibility of the method.

The method was devised specifically to produce inflammation in the hard palate of the rat and probably can be used in any tissue accessible to contact. In the study of a tissue as firm and dense as palate, the injection of an irritant was not desirable because of the very limited dissemination of the stimulus. Further, the restricted size and thickness of this tissue make injection a difficult procedure. In an oral tissue bathed by saliva and other fluids, topical application of an irritant is also difficult and unreliable. Actual surgical incision does not meet the requirements stated above. The application of heat, either directly or by irradiation, is mechanically unfeasible where the specific area is not an outer surface of the animal.

Among the more recent methods, the granuloma-pouch technique of Selye [1] has been widely used. This procedure generates a lesion which is very useful for studies on exudate or histological study of the involved tissue but does not give a tissue sample satisfactory for the chemical analyses desired in this laboratory. The technique of Menkin,[2] involving the injection of turpentine into the pleural cavity of dogs, admirably suited to the study of exudate, suffers the same limitation.

Experimental Study

Site.—Kutuzov and Sicher [3] have found that the hard palate of the rat can be subdivided into four distinct areas: (*a*) the roof of the oral atrium; (*b*) the antemolar region, anterior to the molars; (*c*) the intermolar area, extending between the molars and slightly posterior to the last molars, and (*d*) a small postrugal field, reaching to a transverse terminal ridge, the boundary between the hard and the soft palate. Since the antemolar area of the hard palate was used in this experiment, only this portion will be described in detail.

The antemolar portion shows three straight transversal high rugae, without much differentiation of their surface. The first straight ruga is fused in the midline with the posterior aspect of the incisal papilla, which, in surface view, re-

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National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service, Department of Health, Education, and Welfare.

sembles a clover leaf. The second ruga is separated from the first by an anteroposteriorly wide valley, whose bottom is almost flat. The same is true for the field between the second and third straight rugae and for the field that separates the antemolar from the intermolar rugae. The crests of the second and third straight rugae are fairly sharp and smooth.

Method.—The tissue was stimulated to an inflammatory response by a pulse of radio-frequency current. The signal was generated by an instrument manufactured for clinical electrosurgical procedures, the Radiotome Model STS,* modified for this work by removing several plates of the variable condenser in the output circuit and by rewiring the operating control switch to include a Micro-Flex timer. The stimulating electrode was machined from surgical stainless steel, the part contacting the tissue consisting of a 1×3 mm. rectangular surface. With the apparatus in its final form and the electrode connected to the "coagulating" circuit, a signal of about 3 megacycles at 1000 volts with an intensity variable from 0.1 to 2.0 amp. R. F. was obtained. The desired intensity for any series of treatments was obtained by adjusting the variable condenser (tube intensity control) while a continuous signal was generated with the stimulating electrode in contact with the indifferent electrode. The current intensity was measured with an R. F. ammeter† in series with the stimulating electrode.

Female Sprague-Dawley rats, weighing approximately 150 gm., from the National Institutes of Health stock colony, were anesthetized with pentobarbital sodium (Nembutal), 40 mg/kg. of body weight injected intraperitoneally. A single pulse of 1.00±0.02-second duration was then delivered to the anterior face of the second and third rugae of the hard palate. Recovery from anesthesia was assisted by intraperitoneal injection of picrotoxin, 3 mg/kg., immediately following treatment. At selected time intervals following treatment, the animals were killed by chloroform inhalation, and the antemolar soft tissue of the hard palate was excised immediately for glycogen analysis. The sample was rinsed briefly in distilled water, blotted dry on filter paper, weighed to ±10γ on a Roller-Smith balance, transferred to a centrifuge tube containing 1 ml. of 30% KOH and heated 20 minutes in boiling water to dissolve the tissue. One glycogen determination was made on each palate. Glycogen was precipitated with 2 volumes of 95% ethanol and packed by centrifugation at 2000 rpm for 20 minutes. A modification of the anthrone procedure of Seifter et al.[1] was applied to the drained residue. Killings were timed so that an average of two, and never more than three, minutes elapsed between death of the animal and fixation of the weighed sample in the first step of the analytical procedure. Each of the 12 time intervals was represented by six animals. Untreated animals from the same animal pool were killed at the same time for control data.

Several representative samples for each time interval were prepared for histological study by fixing the entire head in 10% formalin after killing the animal by chloroform inhalation. After two to three days, the palate was excised and cut in the midline. Following dehydration, clearing, and paraffin embedding, multiple sections, 6μ thick, were made from each specimen. These sections were stained with hematoxylin and eosin, the periodic acid-Schiff reagent, and the periodic acid-Schiff reagent after diastase treatment.

Results

It was found that the satisfactory intensity range for creating a standard lesion was between 0.27 and 0.47 amp. R. F. Intensities greater than 0.47 amp. R. F. were so destructive that the entire palate was necrotized. An intensity of 0.40 amp. R. F. consistently produced a lesion confined to the rugae and superficial tissues, which healed in about 14 days. An intensity of 0.27 amp. R. F. produced lesions comparable to those of 0.40 amp. R. F. after 10 hours, but in the earlier periods the lesions were slower to develop and less sharply defined.

Glycogen Analysis

Figure 1 shows the changes in the tissue glycogen at three different intensity levels, each point being the mean of data from six animals. The normal glycogen level, shown by the horizontal broken line in Figure 1, is the mean of data from 45 untreated animals which were killed and analyzed at the same time as the treated animals. The combined mean normal level from the three separate experiments was 0.071%, with a standard deviation of ±0.025, indicating a reasonably low variation due to analytical error and biological variation. An estimation of the reliability of the data can be

* From the Coles Corporation, 1207 Race St., Philadelphia 7.

† Triplet thermocouple type R. F. ammeter, Model 341-T.

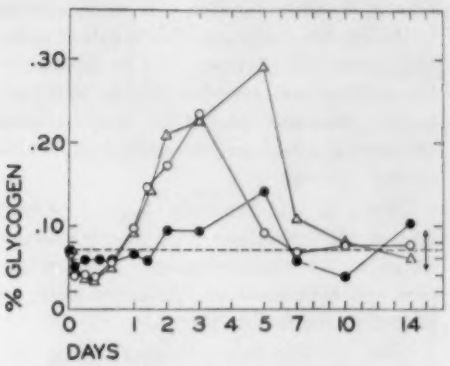


Fig. 1.—Changes in glycogen concentrations at different stimulus intensities. Solid circles, 0.27 amp. R. F.; open circles, 0.40 amp. R. F.; open triangles, 0.47 amp. R. F. Horizontal broken line represents the normal level, with the standard deviation shown as a perpendicular.

obtained from consideration of the coefficients of variation for the points on a single curve. In the experiment at 0.40 amp. R. F. the coefficient of variation was 24% for the 15 normals and ranged from 16% to 48% for the 12 time intervals. The only two values over 40% were at two and five days, points of maximum slope on the curve. The reproducibility of this method was tested in five experiments at separate times at the same current intensity (0.40 amp. R. F.). Although some scatter resulted from comparing single values with mean values, the general correspondence of the five curves in shape and degree was excellent.

Gross Examination

On gross examination nothing was seen at the site of treatment until several hours afterward. The tissue then became increasingly edematous until 16-24 hours after treatment, at which time edema subsided and traces of redness were apparent. From one to three days tissue damage was easily seen, with some necrosis apparent in the lesions resulting from stimulation at the higher intensities. From 5 days onward, healing was observed, with a healthier, if not normal, appearance by 14 days.

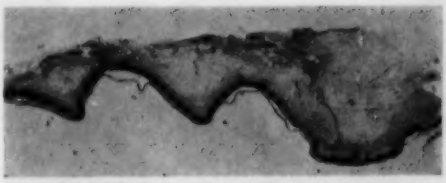
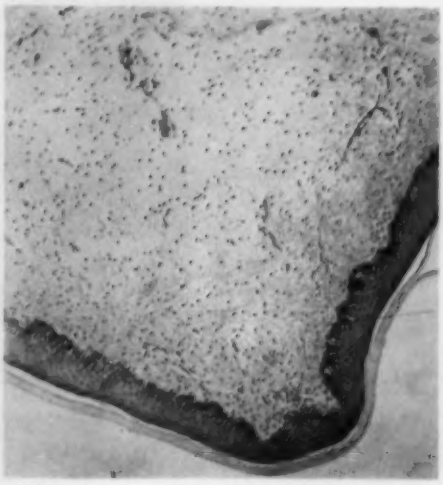


Fig. 2.—A normal antemolar area of rodent hard palate. Reduced to about 69% of mag. × 16.

Microscopic Examination

Description of Normal Antemolar Area of Hard Palate (Figs. 2 and 3).—The bulk of the rugae consisted of a loosely arranged, delicate fibrous tissue. Between the latter tissue and the covering keratinized, stratified squamous epithelium was a lamina propria, made up of dense collagenous fibrous tissue. The rugae rested on a submucosa consisting of adipose tissue and several prominent blood vessels and nerves. Cartilage was found in the first, or most anterior, ruga. Special stains revealed granules of glycogen in minute quantity in the deeper layers of the epithelium, particularly in the rete pegs, directly overlying the crest of the rugae. The time-sequence description of the development and resolution of the induced lesions that follows was derived from specimens treated with an in-

Fig. 3.—Normal ruga at higher magnification, showing fibrous components. Reduced to 80% of mag. × 110.



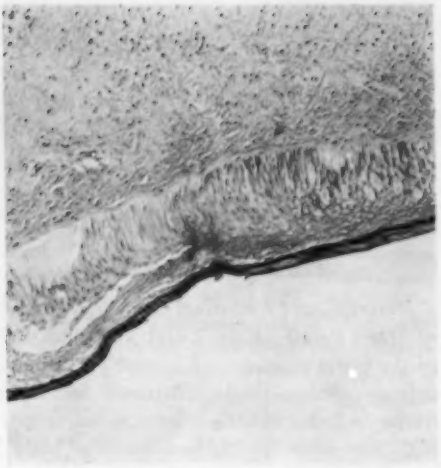


Fig. 4.—Lesion 10 minutes after treatment, showing the small collections of edematous fluid and the stretching of epithelial nuclei. Reduced to about 90% of mag. × 98.

tensity of 0.40 amp. R. F. These observations were found to be reproducible in replicate samples.

Descriptions of Induced Lesions at Various Time Intervals.—Ten-Minute Interval (Fig. 4): Most of the lesions were not confined to the treated surfaces but extended to the opposite surfaces of the adjacent rugae as well. Intercellular and intracellular edema leading to foci of intraepithelial blistering was common. The involved nuclei were stretched perpendicular to the epithelial surface and revealed nuclear fragmentation. Stainable edematous fluid occupied the cleavages between the epithelium and the lamina propria.

Thirty-Minute Interval (Fig. 5): Cleavage of the epithelium with disorganization of its nuclei was prominent. Some dilatation and hyperemia of capillaries were apparent in the lamina propria.

One- to Ten-Hour Interval (Fig. 6): Blister formations had coalesced to involve most of the epithelium of the antemolar area. At first only spotty margination and diapedesis of neutrophilic granulocytes were seen in the lamina propria and submucosa; but with the increasing dilatation and hyperemia of capillaries and the accompanying edema, neutrophilic granulocytes soon appeared in great numbers, some being found in the blisters of epithelium. Hemorrhage within the rugae became manifest toward the end of this period.

Sixteen- to Thirty-Six-Hour Interval (Fig. 7): Neutrophilic granulocytes had increased in number within the blisters. Bacterial growth was first seen within the

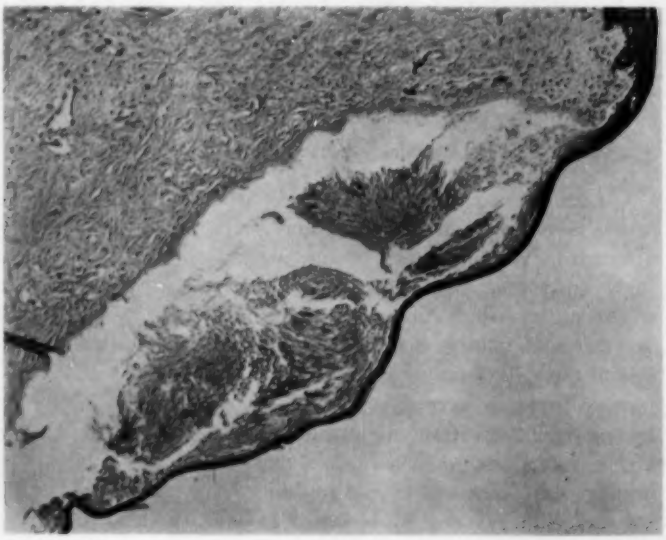


Fig. 5.—Lesion 30 minutes after treatment, showing prominent cleavage of epithelium. Reduced to 96% of mag. × 96.

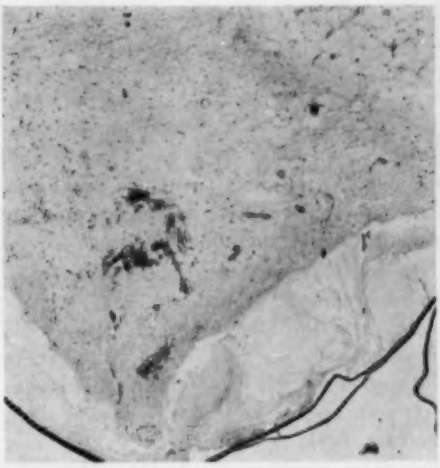


Fig. 6.—Lesion 10 hours after treatment, illustrating massive blister formation, loss of cellular detail, neutrophilic granulocytes within blisters, and hemorrhage in the center of the ruga. Reduced to about 90% of mag. × 63.

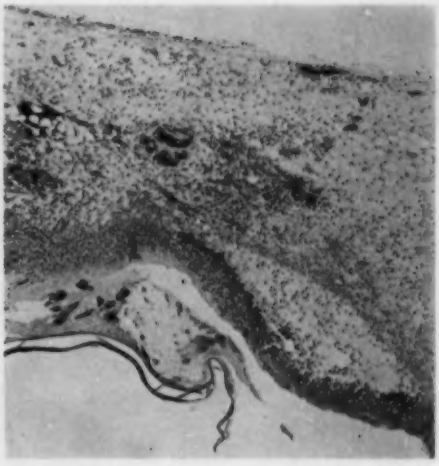


Fig. 7.—Lesion 24 hours after treatment, illustrating the sloughing off of the blistered epithelium, growth of micro-organisms, and accumulation of neutrophilic granulocytes at the surface of the lesion. Reduced to about 90% of mag. × 63.

blisters at 16 hours. By 24 hours most of the blistered epithelium had sloughed off, leaving behind an ulcerated surface, coated with a purulent exudate mixed with colonies of micro-organisms. The crests of the rugae had been flattened.

Three-Day Interval (Fig. 8): A very thick mantle of bacterial growth rested upon the purulent exudate. Proliferation of fibroblasts with mitoses was found in the submucosa, as well as beginning proliferation of epithelium at the margins of the ulcer.

Seven-Day Interval (Fig. 9): A dense proliferation of endothelial cells and fibroblasts now occupied the bulk of the lesion. Fewer micro-organisms were seen with the formation of granulation tissue.

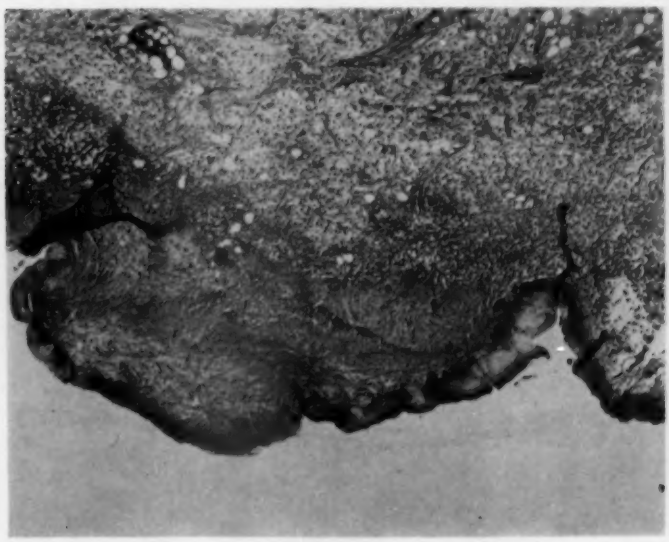


Fig. 8.—Lesion three days after treatment, showing the thick mantle of bacterial growth on the surface of the denuded lesion. Reduced to 96% of mag. × 63.

Fig. 9.—Lesion seven days after treatment, illustrating the dense proliferation of endothelial cells with capillary formation and fibroblasts to form granulation tissue. Note loss of bacterial growth. Reduced to 80% of mag. × 112.

Ten-Day Interval (Fig. 10): Obvious flattening, and sometimes complete loss, of rugae was found. The lesions were partially or completely sealed over with spongiotic epithelium.

Fourteen-Day Interval (Fig. 11): The rugae were missing. The epithelium appeared less spongiotic. Rete pegs were not prominent.

Twenty-One- to Twenty-Eight-Day Interval (Fig. 12): Rete pegs were well formed. An evagination of the previously flattened surface had occurred, probably representing regeneration of a ruga.

Histologic Demonstration of Glycogen.—In the healing of the induced lesion, a slight increase in the amount of glycogen could be seen at the margins of the lesion where the epithelium was proliferating. This was first seen at 36 hours. At 14 days a slight increase could still be seen in the new epithelium covering the lesion. However, any great focal increase in glycogen in the epithelium was usually associated with filtering neutrophilic granulocytes.

Comment

This new experimental technique devised for the study of inflammation involves the application of a rigidly controlled irritating stimulus to a standard tissue sample. In this method the electrode which touches the tissue is not hot and leaves no immediately obvious gross signs following treatment.

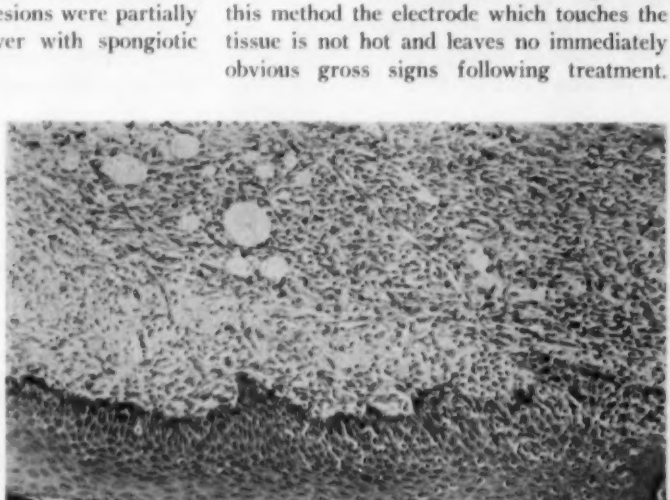


Fig. 10.—Lesion 10 days after treatment, showing new epithelium sealing over the granulation tissue. Note flatness of surface and loss of ruga. Reduced to 96% of mag. × 115.

Fig. 11.—Lesion 14 days after treatment, showing loss of rugae. Reduced to about 69% of mag. × 16.

The variables upon which the degree of inflammatory response depends are (*a*) the intensity of the applied current, (*b*) the contact area of the stimulating electrode, (*c*) the duration of the stimulus, and, presumably, (*d*) the susceptibility of the animal. Of these, the first is controlled by the circuitry of the apparatus; the second, by the design of the electrode, and the third, by an automatic timer. The elucidation of the nature of sensitivity to external stimuli is the objective of future studies, for which this report serves as an introduction. The use of radio-frequency current avoids the introduction of foreign material into the experimental site and is more amenable to precise control of intensity than that of heat alone. Since the primary stimulus is brief (1.0 second), time after treatment may be measured exactly, in terms of minutes, if desired.

The use of the antemolar area of the palate in the rat as an experimental site for the study of inflammation and/or wound healing has several advantages. The triangular palate is easily distinguished from the adjoining buccal mucosa and can be removed simply and rapidly, giving a sample composed of epithelial and connective tissue in constant proportion, free of hair and muscle, and with minimal amounts of fat or neural and vascular tissue. Each palate thus represents a discrete unit which is well suited for analysis of enzymatic activity or chemical composition. The palate is easily accessible for treatment and for observation in the living animal.

The glycogen data are presented primarily as an example of the reproducibility obtained with this technique and as an indication of the tissue response. A more detailed examination of the behavior of glycogen in inflamed tissue will be reported in another publication.[5] Considering the uniformity of the data, the method seems to provide a controlled variable stimulus of reproducible nature.

Summary

A new experimental procedure for the study of the inflammatory response is described. This method consists essentially of the controlled application of radio-frequency current and has been used to produce inflammation in the hard palate of the rat. The advantages of this procedure are discussed, and some preliminary observations are presented.

National Institute of Dental Research (14).

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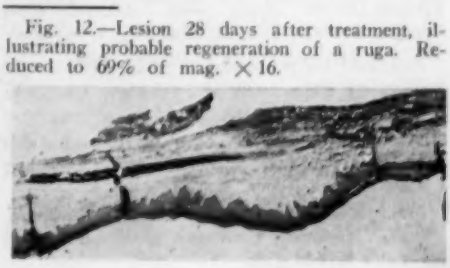
Fig. 12.—Lesion 28 days after treatment, illustrating probable regeneration of a ruga. Reduced to 69% of mag. × 16.



Cancerogenic Effects of Ca^{45} and Sr^{89} in Sprague-Dawley Rats

JOSEPH F. KUZMA, M.D., and GLORIA ZANDER, M.A., Milwaukee

Introduction

The present investigation was designed to study the long-term cancerogenic effects of Sr^{89} and Ca^{45} in the rat. Reports from the Argonne Laboratories showed that bone tumors could be induced in mice by single and monthly intraperitoneal injections of Sr^{89} [1-5] and by single injections of Sr^{89} in dogs,[6,7] rabbits,[1,2] and rats.[1,2] Intravenous injections of Ca^{45} or Sr^{90} producing bone tumors in mice were also reported from the same laboratory.[8] Studies in our own laboratory showed that bone tumors could be produced by Ca^{45} and Sr^{89} in mice [9] and by Sr^{90} in rats [10] by intraperitoneal injections. It has been established, therefore, that bone tumors can be produced in some animals by these radioactive isotopes. However, no data have been published concerning bone-tumor production by Ca^{45} in the rat, and the published data on bone tumor production by Sr^{89} in the rat are meager. Therefore, it seemed particularly advisable to study in detail the long-term cancerogenic effects of Sr^{89} and Ca^{45} in rats allowed to live out their normal life span.

Materials and Methods

Strain of Rats.—Virgin female rats of the Sprague-Dawley strain were obtained from Sprague-Dawley, Inc., in Madison, Wis. They were approximately 3½ months of age when the experiment was begun.

Sr^{89} and Ca^{45}.—Sr^{89} * has a half-life of 53 days and a maximum beta energy of 1.463 mev. Ca^{45} has a half-life of 163 days and a beta energy of 0.254 mev. Both were obtained from the Oak Ridge National Laboratory in solutions of hydrochloric acid which were neutralized with sodium hydroxide and diluted with saline.

Plan of Experiments.—Because the specific activity of the Ca^{45} shipments was rather low, i. e., on the order of 15 to 20 mc. per gram of calcium, it was decided to divide the total dose into 10 injections so as to prevent death from excess of carrier (calcium chloride).

One hundred rats were injected intraperitoneally with 10 consecutive daily doses of Ca^{45} in total doses ranging from 0.1 to 3.5 μc per gram of body weight (Experiment A), while another one hundred were given the same total dose intraperitoneally in 10 monthly injections (Experiment B). Experiments C and D, with Sr^{89}, were set up comparable to those with Ca^{45}. Forty rats were maintained as controls (Table 1).

The animals were maintained on a standard diet, ad libitum, of Rockland rat pellets. The rats were weighed once a month and x-rayed every two months until death in order to detect and study development of bone lesions. Autopsies were performed and tissue sections prepared.

Autoradiography of Bone Tumors.—Autoradiograms † of tumors were prepared from each group of rats in which malignant bone tumors appeared. Part of each tumor was fixed in an alkaline solution of 3 parts 95% alcohol and 1 part 40% neutralized formaldehyde. According to Bélanger,[13]

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* At no time did any of the Sr^{89} shipments contain more than 10% Sr^{90}. Sr^{90} has a half-life of 25 years and a maximum beta energy of 0.61 mev. It decays to Y^{90}, which, in turn, decays, with a half-life of 2.54 days and a maximum beta energy of 2.18 mev, to stable zirconium.

† According to Boyd,[11] the term "autoradiogram" is preferable to "autoradiograph" in designating the result of the technique in which a photographic emulsion is placed in contact with a radioactive specimen.

TABLE 1.—*Plan of Experiments A, B, C, and D*

Ca^{45} or Sr^{89}, Dose, μc/Gm. Body Weight*	Total Dose, μc/Gm. Body Weight	No. of Animals in Each Dose Group
0.35	3.5	20
0.25	2.5	20
0.10	1.0	20
0.05	0.5	20
0.01	0.1	20
Controls		40

Experiment A: Intraperitoneal injections of Ca^{45} in 10 consecutive daily doses (100 animals divided into five groups of 20 each according to dose).

Experiment B: Same as Experiment A, but Ca^{45} given in 10 monthly injections.

Experiment C: Same as Experiment A, but Sr^{89} used instead of Ca^{45}.

Experiment D: Same as Experiment B, but Sr^{89} used instead of Ca^{45}.

* Dose in each of 10 daily or 10 monthly intraperitoneal injections.

this is a good fixative for mineral salts of bone and teeth.

After fixation the bone tumors were dehydrated and embedded in Ward's Bio-Plastic. Thin slices of the tumors were made by cutting with a band saw, followed by grinding and polishing. The smooth cut surfaces were then apposed to x-ray film. After exposure the films were developed in Kodak x-ray developer (at 20 C) for three minutes and fixed for 10 minutes in Kodak acid fixer.

Results

Animal Survival.—All of the 20 rats which were given 3.5μc of Sr^{89} per gram in 10 consecutive days died within six months, and those given 2.5μc per gram in 10 days were dead in nine months.‡ Early Sr^{89} deaths are in a great measure due to bone-marrow destruction. Nine of the rats injected with 3.5μc per gram had developed a bloody crust around the eyes and nose within a few weeks. Anthony and associates [13] made similar observations following injection of toxic doses of Sr^{89} in rats and mice. This condition may be a manifestation of radiostrontium toxicity and, as Ray and co-workers [14] suggest, the development of bloody noses in rats following injection of Sr^{90} indicates increased tendency toward hemorrhage or upper respiratory tract infection.

‡ The $L.D._{50}$/30 days for Sr^{89} when given in a single intraperitoneal injection to rats is slightly less than 5μc per gram.[12]

The rats which were given 2.5 and 3.5μc of Sr^{89} per gram in 10 consecutive days suffered a loss in weight or a depression in weight gain shortly after the injections. The latter group especially was very emaciated before death. There were no significant differences in weight between the controls and the rats given 0.1 to 1.0μc of Sr^{89} per gram in 10 consecutive daily or monthly doses. The Ca^{45}-injected rats showed no particular weight differences from the controls.

Thirteen to fourteen months after the initial injections of Ca^{45} and Sr^{89} pulmonary and abdominal infections caused the death of at least 60 experimental rats and 20 controls. Autopsies revealed "Proteus" abscesses in the lungs, mesentery, and liver. Oxytetracycline (Terramycin) was injected intramuscularly into all of the rats at this time, after which the mortality rate decreased.

Twenty-seven months after the initial injections of Ca^{45} and Sr^{89} the experiments were concluded by killing the surviving rats (one from Experiment A, four from Experiment C, and two controls).

Malignant Bone Tumors.—Tables 2 to 5 summarize the data regarding incidence of malignant bone tumors, latent periods, and survival. The latent period of tumor development is based on the earliest date of detection by x-ray examination followed by histological confirmation. In comparing the survival rates of animals given the highest doses of Ca^{45} with those given the same doses of Sr^{89}, it is obvious that survival is much better in the Ca^{45} animals. However, in those Sr^{89} animals which survived long enough for bone tumors to develop, the incidence of tumors was much higher than in those animals given the same doses of Ca^{45} (for example, 0.10 and 0.05μc per gram per day; 0.35 and 0.25μc per gram per month). Malignant bone tumors appeared in four groups given Sr^{89} and in only three groups given Ca^{45}. Moreover, the minimum and average latent periods of tumor development are shorter in the Sr^{89}

TABLE 2.—*Malignant Bone Tumors: Experiment A: Intraperitoneal Injections of Ca^{45} in Ten Consecutive Daily Doses; Sprague-Dawley Rats*

Daily Dose, μc/Gm. Body Weight	No. of Rats	No. of Rats Which Developed Malignant Bone Tumors	Minimum Latent Period (Mo.) of First Tumor After Injections	Average Latent Period (Mo.) of First Tumor After Injections	Survival		Incidence of Tumors in Rats Which Survived Average Latent Period, %†
					With Tumor No. Surviving Average Latent Period or Dying Sooner with Bone Tumors	Tumor-Free* Average Survival (Mo.) Beyond Beginning of Experiment	
0.35	20	11	9.5	13.9	16	----	68.8
0.25	20	6	10.5	14.2	14	----	42.9
0.10	20	0	----	----	----	16.8	0
0.05	20	0	----	----	----	18.5	0
0.01	20	0	----	----	----	18.8	0
Controls	40	0	----	----	----	16.8	0

*"Tumor-free" means free of malignant bone tumors.
†In this Table and in Tables 3-5, rats with bone tumors which did not survive the average latent period are included.

TABLE 3.—*Malignant Bone Tumors: Experiment B: Intraperitoneal Injections of Ca^{45} in Ten Monthly Doses; Sprague-Dawley Rats*

Monthly Dose, μc/Gm. Body Weight	No. of Rats	No. of Rats Which Developed Malignant Bone Tumors	Minimum Latent Period (Mo.) of First Tumor After Initial Injection	Average Latent Period (Mo.) of First Tumor After Initial Injection	Survival		Incidence of Tumors in Those Rats Which Survived Average Latent Period, %
					With Tumor No. Surviving Average Latent Period or Dying Sooner with Bone Tumors	Tumor-Free* Average Survival (Mo.) Beyond Beginning of Experiment	
0.35	20	7	10.0	15.6	12	----	58.3
0.25	20	0	----	----	----	18.7	0
0.10	20	0	----	----	----	14.2	0
0.05	20	0	----	----	----	16.2	0
0.01	20	0	----	----	----	13.5	0
Controls	40	0	----	----	----	16.8	0

*Free of malignant bone tumors.

TABLE 4.—*Malignant Bone Tumors: Experiment C: Intraperitoneal Injections of Sr^{89} in Ten Consecutive Daily Doses; Sprague-Dawley Rats*

Daily Dose, μc/Gm. Body Weight	No. of Rats	No. of Rats Which Developed Malignant Bone Tumors	Minimum Latent Period (Mo.) of First Tumor After Injections	Average Latent Period (Mo.) of First Tumor After Injections	Survival		Incidence of Tumors in Those Rats Which Survived Average Latent Period, %
					With Tumor No. Surviving Average Latent Period or Dying Sooner with Bone Tumors	Tumor-Free* Average Survival (Mo.) Beyond Beginning of Experiment	
0.35	20	0	----	----	----	3.3	0
0.25	20	0	----	----	----	5.8	0
0.10	20	3	8.0	8.5	5	----	60.0
0.05	20	10	8.5	14.5	16	----	62.5
0.01	20	0	----	----	----	19.0	0
Controls	40	0	----	----	----	16.8	0

*Free of malignant bone tumors.

animals. These observations show that Sr^{89} is a more effective cancerogen than Ca^{45}.

Distribution of Malignant Bone Tumors.—Distribution of malignant bone tumors is tabulated in Table 6. Both Ca^{45} and Sr^{89} animals developed tumors chiefly in the hindlimbs. However, only Ca^{45} rats developed tumors of the spine and pelvic bones. Explanation of this variance in tumor distribution is lacking. No malignant bone tumors appeared in the control animals. Multiple malignant bone tumors, which appeared almost simultaneously (within one month of one another), developed in both the Sr^{89} and the Ca^{45} animals, as shown in Table 7. Multiple malignant bone tumors appeared in 6 of 24 Ca^{45} and in 31 of 46 Sr^{89} animals with malignant bone tumors.

TABLE 5.—*Malignant Bone Tumors: Experiment D: Intraperitoneal Injections of Sr^{90} in Ten Monthly Doses; Sprague-Dawley Rats*

Monthly Dose, μc/Gm. Body Weight	No. of Rats	No. of Rats Which Developed Malignant Bone Tumors	Minimum Latent Period (Mo.) of First Tumor After Initial Injection	Average Latent Period (Mo.) of First Tumor After Initial Injection	Survival		Incidence of Tumors in Those Rats Which Survived Average Latent Period, %
					With Tumor No. Surviving Average Latent Period or Which Died Sooner with Bone Tumors	Tumor-Free* Average Survival (Mo.) Beyond Beginning of Experiment	
0.35	20	19	6.5	9.0	19	----	100.0
0.25	20	14	8.0	9.9	17	----	82.4
0.10	20	0	----	----	----	13.5	0
0.05	20	0	----	----	----	16.0	0
0.01	20	0	----	----	----	16.0	0
Controls	40	0	----	----	----	16.8	0

*Free of malignant bone tumors.

TABLE 6.—*Distribution of Malignant Bone Tumors*

Original Site of Bone Tumors	Experiment A Ca^{45} in 10 Consecutive Daily Doses	Experiment B Ca^{45} in 10 Monthly Doses	Experiment C Sr^{90} in 10 Consecutive Daily Doses	Experiment D Sr^{90} in 10 Monthly Doses	Controls
Left foreleg	1	1	0	5	0
Right foreleg	2	2	1	7	0
Left hindleg	3	2	7	30	0
Right hindleg	8	5	11	30	0
Skull	1	0	0	1	0
Spine	4	0	0	0	0
Pelvis	2	0	0	0	0

TABLE 7.—*Multiple Malignant Bone Tumors*

Experiment A Daily Dose of Ca^{45}, μc/Gm. Body Weight	Experiment B Monthly Dose of Ca^{45}, μc/Gm. Body Weight	Experiment C Daily Dose of Sr^{90}, μc/Gm. Body Weight	Experiment D Monthly Dose of Sr^{90}, μc/Gm. Body Weight	No. of Rats with Multiple Malignant Bone Tumors	Average No. of Primary Malignant Bone Tumors
0.35	----	----	----	2	2.0
0.25	----	----	----	2	2.0
----	0.35	----	----	2	2.5
----	----	0.10	----	2	2.5
----	----	0.05	----	3	2.0
----	----	----	0.35	13	2.5
----	----	----	0.25	13	2.6

TABLE 8.—*Benign Bone Tumors*

Experiment A Daily Dose of Ca^{45}, μc/Gm. Body Weight	Experiment B Monthly Dose of Ca^{45}, μc/Gm. Body Weight	Experiment C Daily Dose of Sr^{90}, μc/Gm. Body Weight	Experiment D Monthly Dose of Sr^{90}, μc/Gm. Body Weight	Control Rats with Benign Bone Tumors	No. of Rats with Benign Bone Tumors	Location of Tumors	Latent Periods After Initial Injection of Ca^{45} or Sr^{90}, Mo.
0.35	----	----	----	----	1	Left foreleg	17.0
0.25	----	----	----	----	1	Right hindfoot and ankle	18.5
----	0.35	----	----	----	1	Right heel	11.5
----	0.10	----	----	----	1	Left hindfoot	7.5
					(4 tumors)	Tail	13.5
						Right wrist	13.5
						Left wrist	13.5
----	0.05	----	----	----	1	Left heel	18.5
----	0.01	----	----	----	1	Left hindleg	11.5
----	----	0.01	----	----	1	Right hindfoot	17.00
----	----	----	None	----	0		
----	----	----	----	None	0		

The greatest number of tumors in any animal was five (Sr^{89}).

Metastases.—Pulmonary metastases were the commonest in both Sr^{89} and Ca^{45} groups and were present in slightly more than 50% of the animals with malignant bone tumors. Other organs were involved only occasionally, in the following order: spleen, heart, liver, lymph nodes, and mesentery. The Sr^{89}-induced bone tumors are generally less differentiated and more invasive than those induced by Ca^{45}. This, however, is not reflected in incidence of metastases. Ca^{45} animals have a somewhat longer life span, and metastases occurring late in life may balance the number appearing earlier in the Sr^{89} animals.

Benign Bone Tumors.—The data on benign bone tumors is presented in Table 8. Seven rats were so affected, six with Ca^{45}, one with Sr^{89}, and none of the controls. The average latent period for these tumors is 14.2 months. Two of the Ca^{45} rats also had a malignant bone tumor. Benign bone tumors, appearing as exostoses or hyperostoses, were chiefly present at the ends of the extremities, rather than in the long bones. Malignant tumor development from such hyperostosis occurred in one animal. If the life span were longer, a higher incidence of malignant change in the hyperostoses could be expected in these lesions of the small bones. In the Ca^{45}-induced malignant tumors many areas of quite mature bone were present, but these are not classified as benign tumors.

Snout Tumors.—Seven of the Sr^{89}-injected rats developed snout soft-tissue tumors—the earliest at 9 months and the latest at 22 months, with the average of 12.9 months. All tumors were squamous-cell carcinomas and were found only in the Sr^{89} animals, namely, one in the group given 1μc per gram in 10 days, two given 0.35μc per gram per month, one given 0.5μc per gram in 10 days, one given 0.25μc per gram per month, and two given 0.1μc per gram per month. Five of the seven snout tumor rats were in groups with malignant bone tumors; two were in groups without. Four of the seven rats with snout carcinoma also had osteogenic sarcomas of the extremities, while two of the three without bone sarcoma were from a dose group in which no bone tumor developed. The squamous-cell carcinoma involved the nose or forepart of the mouth and developed from the mucosal surface. The mechanism of development is not clear at the present time, but it appears to be an effect of radioactive strontium, as none developed in the controls or in the Ca^{45}-injected rats. As mentioned above, high doses of Sr^{89}, that is, 3.5μc per gram in 10 days, may produce bleeding, crusting, and inflammatory reaction about the eyes and nose, and such has been attributed to radiostrontium toxicity. Such rats do not survive the minimum latent period of tumor development. On the other hand, the development of squamous-cell carcinoma, in such an area, in rats given smaller doses of Sr^{89} strongly suggests a cause-and-effect relationship.

Other Tumors.—Nearly 15% of these Sprague-Dawley rats developed breast tumors (fibroadenoma). There was no significant difference of incidence in any groups, that is, controls, those given Ca^{45} or Sr^{89} and those with or without bone tumors. Only Ca^{45} rats without bone tumors developed any other type of neoplasm—namely, two, soft-tissue reticulum-cell sarcomas; one, rhabdomyosarcoma, and one, possible leukemia.

Fractures.—Heller [15] reported frequent fractures of the long bones in rats given Sr^{89}. Heller's rats were considerably younger than ours at the time of Sr^{89} injection, and this may explain the lack of agreement, since in our animals no spontaneous fractures were observed in any group. However, three Sr^{89} animals had pathologic fractures in a bone with manifest malignant tumor. In these three the diagnosis was made radiographically, but many more fractures were evident upon gross and histologic examination of other tumors, especially in the Sr^{89}-injected animals.

Pathology of Malignant Bone Tumors.—A. Gross Findings: Tumors were generally distributed in the metaphyses of the long bones, chiefly in the distal end of the femur and the proximal end of the tibia. Generally the pattern of tumors was one of two types: (1) a fleshy, grayish-pink, rather soft formation speckled with hemorrhages and necrosis, and (2) a milkier-white, dull tumor with varying degrees of granularity and grittiness. In general, many of the Sr^{89} tumors were of the first type, while the second type characterized the Ca^{45} tumors. Soft-tissue invasion occurred in both but was most evident in the Sr^{89} tumors.

B. X-Ray Appearance: Radiostrontium (Sr^{89}). The earliest changes evident on x-ray examination were generally those of punched-out or mottled rarefaction of the metaphysis, beginning in the medullary canal, destroying the cancellous pattern, and eventually thinning the cortex from the internal aspect. This was followed shortly by dissolution of the cortex and development of soft-tissue tumor.

Radiocalcium (Ca^{45}). The pattern was that of a chalky-appearing increased density of the metaphysis with loss of distinction between medullary canal and cortex. The extension of tumor formation on the outside of the bone produced a granular density, usually showing a fuzzy, spiculated periphery. In some areas, however, the peripheral outline was rather sharp.

In many the tumor pattern, as revealed by x-ray, was not distinctive, but in general Sr^{89} produced a more rapid change, characterized by mottled rarefactions, cortical thinning, and pathologic fractures, while Ca^{45} gave obscuring increased density.

C. Histology: The basic pattern in all cases was an osteogenic sarcoma, in various degrees of differentiation. The variations of patterns might be summarized as follows: (1) a highly and delicately vascularized "fibroblastic" pattern, which very closely mimicked an angiosarcoma; (2) a more definitely fibrous pattern, less intensely vascularized and showing thin threads of osteoid between the cells; (3) variation of the fibrous and osteoid pattern with zones of myxomatous tissue and transitions into distant hyaline cartilage; (4) clear-cut bone-producing tumor of "mature" character, in some instances closely resembling an exostosis or "osteoma"; (5) a very bizarre anaplastic pattern with great numbers of irregular giant cells, many mitoses, variable amounts of osteoid and bone, and considerable necrosis; (6) a highly cellular type of formation, which had a close resemblance to a reticulum-cell sarcoma. Identification of these histologic patterns as osteogenic sarcoma is based on finding some evidence of osseous differentiation either in the primary or in its metastasis. A very wide range of differentiation from anaplasia to almost mature bone was seen in many tumors and also in parts of the same tumor. Lack of differentiation and blood-vessel invasion was more evident in the Sr^{89} tumors. The range of the histologic variation in the metastasis is similar to that of the primary lesion.

D. Autoradiographic Findings: As previously mentioned, autoradiograms were made of tumors from each group in which malignant bone tumors appeared. The data can be summarized as follows:

1. The localization of Ca^{45} in Ca^{45}-induced tumors did not differ significantly from the localization of Sr^{89} in Sr^{89}-induced tumors.

2. In some cases where the tumor was heavily calcified, a considerable concentration of radioactivity was evident within the tumor tissue. When the outer part of the tumor was soft, the periphery showed little or no radioactivity.

3. In some tumors the bone of origin was completely obscured in the autoradiogram. In others the outline of the bone of origin was still intact.

4. Pathologic fractures of bone within bone tumors occurred in three Sr^{89}-induced tumors, while in Ca^{45}-induced tumors there were none. Consequently, fragments of

fractured bone were evident in autoradiograms of these Sr^{89} tumors.

5. Sr^{89}, because of its high maximum energy of 1.463 mev, gave more diffuse autoradiograms than Ca^{45}.

Comment

Sr^{89}, having a beta energy of 1.463 mev and a half-life of 53 days, is a bone-seeking isotope which is highly effective in producing osteogenic sarcomas in the rat. A given total dose of the isotope divided into 10 consecutive daily injections is more effective than the same total dose given in 10 monthly injections, provided, however, that the animals given daily injections survive. For example, a total of 1.0 and 0.5 μc per gram in 10 daily doses produced an incidence of malignant tumors of at least 60%, but the same dose in 10 monthly injections produced no bone tumors. On the other hand, a greater total dose can be given in 10 monthly injections than in 10 daily injections; that is, when a total of 3.5 μc of Sr^{89} per gram was given in 10 consecutive days, there were no survivors beyond six months, while there was 100% survival at seven months when the same dose per gram was given in 10 monthly injections.

Ca^{45}, having a beta energy of 0.254 mev and a half-life of 163 days, is also effective in producing osteogenic sarcomas in the rat. However, it is not as effective as Sr^{89}. For example, 10 daily injections of 0.35 μc of Ca^{45} per gram is about as effective as 0.05 μc of Sr^{89} per gram administered similarly. This is related to its mev, that is, 0.254 for Ca^{45}, as compared with 1.463 for Sr^{89}. When the same doses of Ca^{45} and Sr^{89} are given in monthly injections, a greater cumulative dose can be built up with Ca^{45} than with Sr^{89} because the half-life of Ca^{45} is approximately three times that of Sr^{89}. However, since Ca^{45} is a weak beta emitter, it does not cause as high an incidence of tumors as does Sr^{89} in monthly injections. In fact, acute killing (of mice) cannot be accomplished by very high doses of Ca^{45}, that is, 49.6 μc per gram.[18] Biologic tumor effects, therefore, are related to the mev of Ca^{45} and Sr^{89}. Those of our animals that were given Ca^{45} in monthly injections and that did not develop bone tumors had an average survival of 15 months, whereas the average latent period in those rats that developed bone tumors after monthly injections of Ca^{45} was 15.6 months (Table 3). If the life span were considerably longer, perhaps more tumors might develop, even in the lower dose groups.

It has been stated that the latent period for tumor appearance is quite unrelated to the total dose of the isotope administered. This appears to be generally true under the conditions of these experiments. However, in those experiments in which more than one dose group of animals developed bone tumors, there is a tendency for some lengthening of the latent period with decreasing dose (Tables 2, 4, and 5). Any considerable lengthening of the latent period is, however, limited by the life span of the animal.

Sites where malignant tumors develop show, in the early phases, overt severe injury with Sr^{89} but much less conspicuous changes with Ca^{45}. In fact, the progressive bone repair of Ca^{45} injury appears rather benign in many areas of otherwise frank malignancy. It appears that the Ca^{45} osteogenic sarcoma has a "benign" phase for some significant period of time. In the Sr^{89}-induced tumors this is not quite the same, and it appears that the borderline between repair and neoplasia is much sharper and narrower.

Benign tumors (exostoses) are particularly associated with Ca^{45} (Table 8). The average latent period of exostoses is about the same as osteogenic sarcoma in the Ca^{45} animals. The life span of the animals, however, is not sufficiently long thereafter to allow the benign exostosis to undergo malignant change in most cases. However, finding areas of histologic malignancy in anatomic association with exostosis suggests that these "benign" mature-appearing repair exostoses maintain considerable potential to develop malignancy. In Sr^{89} animals bone-

injury repair of exostosis magnitude usually does not develop, and the progression of repair into malignancy takes place more rapidly, and without a conspicuous intermediate phase of "mature" bone formation (Tables 4 and 5).

The development of snout tumors was unexpected and apparently has not been reported previously with Sr^{89}. All such tumors were squamous-cell carcinomas; all were in animal groups given Sr^{89}, and all appeared in an area known to be affected by radiostrontium toxicity. Experiments are now in progress to study salivary and lacrimal glands for content and excretion of Sr^{89} and to test the hypothesis that the mucosa so closely applied to Sr^{89}-burdened bone tissue in this anatomic area suffers radiation-induced carcinoma after prolonged exposure. Ca^{45} apparently is not of sufficient energy to so influence squamous epithelium in this area.

Conclusions

Ca^{45} and Sr^{89} produce malignant bone tumors in the rat after intraperitoneal injection (10 daily or 10 monthly administrations), and Sr^{89} is the more effective of the two.

Ca^{45}- and Sr^{89}-induced osteogenic sarcoma is related to radiation injury-repair-neoplasia and appears chiefly at the ends of long bones, where growth activity and concentration of the isotope are greatest.

The tumors are osteogenic sarcomas, in varying degrees of differentiation. The majority are found in the lower limbs, that is, the distal end of the femur and the proximal end of the tibia, but only Ca^{45} produced tumors of the spine and pelvis.

Benign bone tumors, i. e., exostoses, occurred in one Sr^{89} rat and in six Ca^{45} animals, appearing usually in the smaller bones of the extremities, notably the foot.

Squamous-cell carcinoma may develop in areas where squamous epithelium is closely approximated to bone burdened with Sr^{89}. This relationship is best seen in the anatomic region of the nose and mouth.

No benign or malignant bone tumors developed in control rats (Sprague-Dawley strain).

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Aortitis with Aortic Valve Insufficiency in Rheumatoid Arthritis

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The association of cardiac valvular lesions and joint disease has been recognized for some time. However, most authorities have been of the opinion that these lesions were due to rheumatic fever. In the past few years several authors have called attention to aortic lesions in patients with typical rheumatoid arthritis, some of whom also had ankylosing spondylitis. Hufnagel's recent report that 5% of patients with aortic insufficiency have ankylosing spondylitis would indicate that the association is not an unusually rare condition.[1]

In 1941 Baggentoss and Rosenberg first reported on cardiac lesions in association with chronic rheumatoid arthritis. One of their patients had multiple valvular, pericardial, and aortic lesions.[2] In a later paper, in 1944, they added a second case having aortic nodular lesions.[3] Raven, Weber, and Price[4]; Gruenwald[5]; Bywaters,[6] and Pirani and Bennett[7] reported similar cases. Bauer, Clark, and Kulka[10] reported five cases without detailed pathological descriptions. Others have mentioned similar lesions but have not reported their findings in detail.[8-15] The information given does not in all instances specify the presence of rheumatoid spondylitis.

A patient with rheumatoid spondylitis and peripheral joint involvement was recently studied at the West Side Veterans Administration Hospital, and the diagnosis of aortic valvular insufficiency due to systemic rheumatoid disease was made prior to death. Since detailed pathological findings have been reported in only a few cases, we believe the findings in our case warrant this report.

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West Side Veterans Administration Hospital; Chicago Medical School, and University of Illinois College of Medicine.

Report of a Case

Clinical History

A 38-year-old white man was admitted to the West Side VA Hospital for the third time on Nov. 17, 1955, because of cardiac failure. Orthopnea, peripheral edema, and angina pectoris were first noted during the early months of 1955, although minor exertional dyspnea had been experienced for five years. There was no history of hypertension, syphilis, or myocardial infarction.

In 1944, while a prisoner of war, polyarthritis involving the ankles, knees, and hips developed and continued until an episode of hepatitis in 1945 brought about a three months' remission of his arthritis. During 1946 and 1947 he had a number of exacerbations with partial remissions. In 1946 he was first told he had a cardiac murmur. He was apparently well from 1948 until March, 1954, when he was admitted to the West Side VA Hospital because of a recurrence of his arthritis, which involved the right ankle, shoulder, and low back. Typical cardiac and peripheral findings of aortic insufficiency, in addition to restriction of chest expansion, limitation of back motion, and roentgenographic findings of rheumatoid arthritis of the sacroiliac joints, were obtained. There were also swelling and tenderness at the insertion of the right tendo achilles. Following x-ray therapy to the spine he was discharged. A year later, on Aug. 8, 1955, he was again hospitalized, because of congestive heart failure. He improved somewhat but was never again in complete cardiac compensation. After a month he left the hospital but within two months was admitted for the third time, in severe cardiac failure.

Physical Examination

The patient was chronically ill, afebrile, and in moderate respiratory distress. Moist rales were heard over both pulmonary bases, posteriorly. The blood pressure was 140/30 mm. Hg; the pulse

rate was 94 a minute, and the cardiac rhythm was regular. The apical impulse was in the left anterior axillary line. Systolic and diastolic murmurs were present in the aortic area. The liver was tender and extended 3 cm. below the right costal margin, and the spleen was felt just below the left costal margin. Both lower extremities were moderately edematous. The findings of rheumatoid spondylitis were unchanged. There was no evidence of active peripheral rheumatoid arthritis.

Laboratory and Special Examinations

Serial electrocardiograms showed left ventricular hypertrophy with a first-degree A-V block. A roentgenogram of the chest demonstrated cardiac enlargement, mainly of the left ventricle. The hemoglobin was 8 gm. per 100 cc.; hematocrit, 25%, and white blood cell count, 6300 per cubic millimeter, with 66% neutrophils, 29% lymphocytes, 2% monocytes, and 3% eosinophils. The nonprotein nitrogen determination was 112 mg. per 100 cc., and the creatinine was 2.2 mg. per 100 cc. The total serum protein was 6.8 gm., albumin 3.6 gm., and globulin 3.2 gm., per 100 cc. The cardiolipin microflocculation test for syphilis was negative. Several blood cultures were negative. Albumin and bile were found in the urine, and on microscopic examination there were 8 white blood cells and 2 red cells per high-power field.

Course in Hospital

In addition to severe cardiac failure the patient complained of abdominal pain, nausea, and vomiting. Subacute bacterial endocarditis was suspected, and penicillin therapy was started. Five days later, on Nov. 22, he developed acute cardiac insufficiency and died.

Autopsy

The body was that of an asthenic and poorly nourished white man, measuring 5 ft. 10 in. (177.8 cm.) in length and weighing 160 lb. (72.6 kg.). No subcutaneous rheumatoid nodules or deformities of the peripheral joints were present.

Internal Examination

The peritoneal cavity was found to contain approximately about 150 cc. of clear, straw-colored fluid. The lower margin of the left lobe of the liver was 5 cm. below the xiphoid process, and the lower margin of the right lobe extended 3 cm. below the costal margin at the right anterior axillary line. The spleen extended 2 cm. below the costal margin at the left anterior axillary line. No pleural adhesions were found, and the pleural cavities and the pericardial sac each contained about 75 cc. of clear yellow fluid.

The heart was enlarged and weighed 700 gm. The epicardial surface was smooth, and the subepicardial fat was decreased. The myocardium was pale and firm, and no evidence of scarring was found. The wall of the right ventricle near the septum measured 0.4 cm., while that of the left ventricle at the mitral valve measured 2.0 cm. in thickness. The tricuspid valve leaflets were thin, and the ring measured 13.5 cm. in circumference. The pulmonic leaflets were not remarkable, and the ring measured 6.5 cm. in circumference. The leaflets of the mitral valve were slightly thickened, as were the chordae tendineae, and the ring of the mitral valve measured 12.5 cm. in circumference.

The important findings were located in the root of the aorta and the aortic valve, as shown in Figures 1 and 2. The aortic valve leaflets were thickened, with inverted, rolled edges and moderate fusion of the commissure between two of the semilunar leaflets. The aortic sinuses were very deep. The proximal segment of the aorta with the aortic root, measuring 7 cm. in length, showed marked dilatation of the aortic wall, which

Fig. 1.—Dilated left ventricle, deformed aortic valve, and aortitis of the root of the aorta.

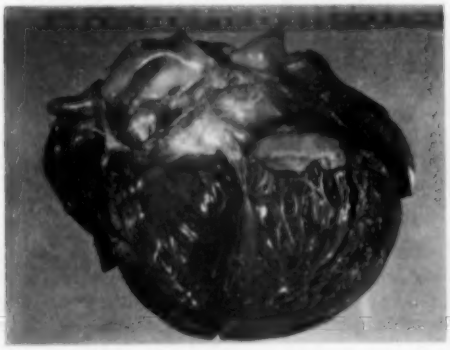

Fig. 2.—Aortic valve with inverted rolled margins of the semilunar leaflets. Root of the aorta with aneurysmal outpouchings and wrinkling of the intimal lining.

measured up to 10.5 cm. in circumference at the midportion of this segment and 9.5 cm. in circumference at the aortic valve ring. The aortic wall of this segment varied greatly in thickness and measured from 4 mm., at the aortic ring, to 2 mm., at the midportion of this segment. Several small aneurysmal outpouchings, which measured up to 1.0 cm. in diameter, were present in the wall, while the transverse wrinkling of the intimal lining gave this surface a "tree-bark" appearance, similar to that described in syphilitic aortitis. These changes of the aorta extended proximally into the sinuses of the aortic valve, and here the orifices of the coronary arteries were displaced distally above the leaflets of the aortic valve. About 3 cm. above the level of the valve these changes in the aortic wall ended abruptly, and the remainder of the ascending aorta, the arch, and the descending aorta appeared normal. No abnormalities were seen in the coronary arteries.

Fig. 3.—Focal necrotic lesion (rheumatoid) of the media of the root of the aorta with similar smaller lesions in the adjacent areas. Hematoxylin-eosin stain; reduced to 92% of mag. × 150.

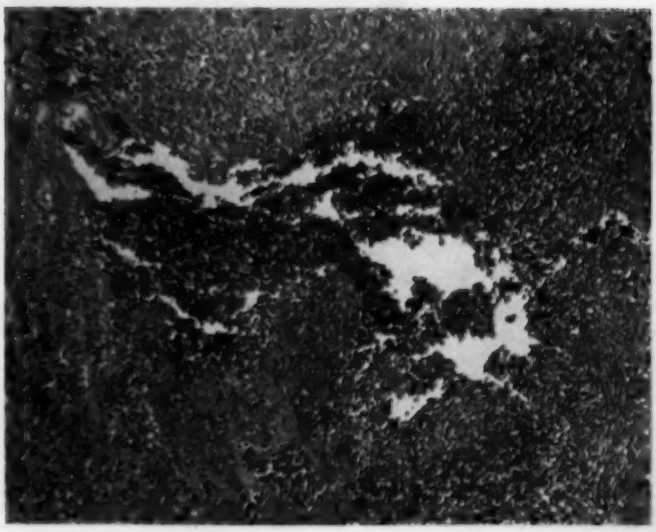

Fig. 4.—Focal necrotic lesion (rheumatoid) of the media of the root of the aorta with polymorphonuclear leukocytic infiltration. Hematoxylin-eosin stain; reduced to 92% of mag. × 150.

The lungs presented moderate edema and passive hyperemia. The liver showed marked chronic passive hyperemia. The spleen was hyperemic, and on cut section prominent Malpighian bodies were noted. The remaining parenchymatous organs, gastrointestinal and genitourinary tracts, were hyperemic.

Microscopic Examination

Serial sections of the aorta showed at several levels focal necrotic lesions of the media surrounded by marked polymorphonuclear leukocytic infiltrations (Figs. 3 and 4). The same microscopic sections prepared with elastic tissue stains (Weigert's method) showed marked fragmentation of elastic fibers in the areas of necrosis (Fig. 5). The same microscopic sections prepared with reticulum tissue stains (Gomori's method) showed preservation of the reticulum fibers with morphological evidence of incomplete necrosis of these lesions (Fig. 6). Special staining procedures (periodic acid-Schiff [PAS], Ziehl-Neelsen, Levaditi, and Brown-Brenn stains) revealed no pathogenic organisms in the areas of necrosis. Microscopic sections of the root of the aorta adjacent to the necrotic lesions showed extensive inflammatory changes about the small blood vessels of the media. These consisted of lymphocytic infiltrations with occasional plasma cells and eosinophils.

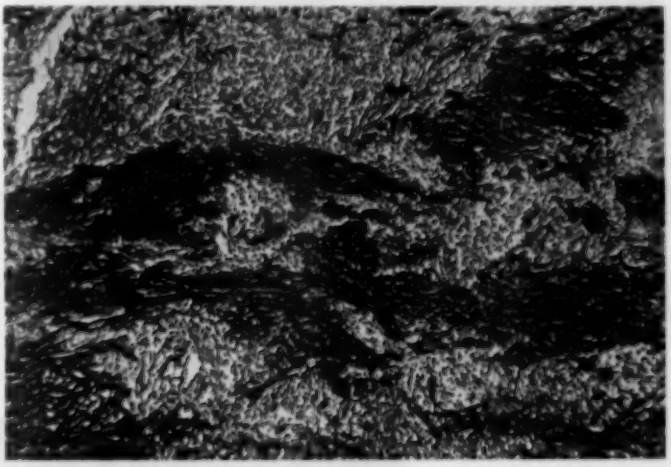

Fig. 5.—Focal necrotic lesion (rheumatoid) of the same area as Figure 4, showing marked elastic tissue fragmentation. Weigert's elastic tissue stain; reduced to 62% of mag. × 300.

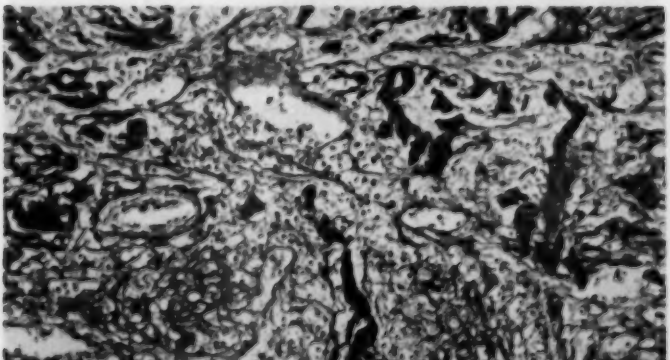

Fig. 6.—Focal necrotic lesion (rheumatoid) of the same area as Figure 4, showing preservation of the reticulum. Gomori's reticulum stain; reduced to 62% of mag. × 500.

Other areas presented lesions resembling the granulomatous nodules of rheumatoid arthritis. The remaining microscopic sections of the root of the aorta showed extensive areas of fibrosis with hyalinization in the media. These were arranged in a whorl-like pattern. Perivascular lymphocytic infiltration of the vasa vasorum of the adventitial was also seen. Similar whorl-like fibrotic areas were present in the mitral valve. The myocardium showed slight perivascular interstitial fibrosis. No Aschoff nodules were present in any part of the heart. Sections from other segments of the aorta showed no noteworthy changes.

The liver presented marked passive hyperemia, with central atrophy and necrosis in many lobules. There was a moderate degree of fatty change. The lungs presented the findings of edema and chronic passive hyperemia. The kidneys showed small focal areas of perivascular lymphocytic and polymorphonuclear leukocytic infiltrations about the small-sized blood vessels and capillaries. The glomeruli were cellular and infiltrated with occasional polymorphonuclear leukocytes. The spleen had distinct lymphocytic hyperplasia of Malpighian bodies and changes of chronic passive hyperemia. The gastrointestinal tract, adrenals, pancreas, prostate, testes, and thyroid gland showed no noteworthy changes. The joints and bones were not examined microscopically.

Comment

In 1940 Bennett, Feller, and Bauer,[16] in a comparative study of rheumatoid and rheumatic subcutaneous nodules, gave a clear morphological description of both lesions and made the statement that they differ from each other as a tubercle differs from a gumma. Since that time an increasing number of reports of rheumatoid arthritis with general rheumatoid manifestations, particularly cardiac lesions, have appeared in the medical literature. Two well-documented cases of cardiac lesions of rheumatoid arthritis were reported by Baggentoss and Rosenberg in 1944.[3] They classified these lesions as "rheumatic" and did not call them "rheumatoid," since they felt that a strict separation of rheumatic and rheumatoid lesions could not be made. In 1948 Raven, Weber, and Price[4] reported a case of rheumatoid arthritis with necrobiotic rheumatoid nodules of the myocardium and similar nodules of the lungs, larynx, spleen, and skeletal muscles. A similar case was reported the same year by Gruenwald.[5] There were rheumatoid nodules of the tricuspid valve, right atrium, lungs, and spleen. Bywaters[6] first attempted to classify cardiac lesions as seen in patients with rheumatoid arthritis. He reported two cases of rheumatoid heart disease and called particular attention to the similarity of chronic exudative inflammatory changes of the synovial membranes and pericardium. Specific rheumatoid granuloma formation was not seen. He also reported the first well-documented case with rheumatoid nodular lesions involving the root of the aorta. Pirani and Bennett,[7] in 1951, reported a patient with rheumatoid arthritis who had chronic arthritis and a deformity of the aortic valve. Similar cases of aortitis of the root of the aorta have been reported or mentioned by others,[8-11,13-15] and the relationship to rheumatoid arthritis has been proposed.

Acute aortitis of the root of the aorta in rheumatoid arthritis is rare. The four well-documented cases reported in the literature[2,5-7] did not have acute lesions. Cases with acute aortic lesions have been mentioned by various authors,[10,11,15] but these were not described in detail.

The most characteristic feature of this acute rheumatoid lesion is the incomplete type of necrosis of the aortic wall seen in our case. Focal fragmentation of the elastic tissue of the aorta distinguishes this lesion from that of syphilitic aortitis, in which the elastic tissue is diffusely involved. Aschoff bodies, marked "onion-skin-like" perivascular fibrosis, and focal regions of fibrinoid degeneration were not found, and the lesions of rheumatic fever were excluded on this basis. The findings were not those of

syphilis. We feel, as do many others,[1,4-7,10-12,14,15] that rheumatoid lesions of the root of the aorta can be differentiated from those of rheumatic fever.

Summary

An unusual type of aortitis with aortic valvular insufficiency occurring in a 38-year-old man with rheumatoid spondylitis is presented. The dilated root of the aorta showed several small aneurysmal outpouchings and marked wrinkling of the intimal lining. The rolled aortic leaflets were inverted, and the aortic ring was also dilated. The microscopic findings were those of necrosis of tissue with focal fragmentation of the elastica, preservation of the reticulum, and cellular infiltration. These findings are interpreted as representing a modification of the necrobiotic lesions of rheumatoid arthritis. Histologically, they can be differentiated from the lesions of syphilis and those of rheumatic fever. The described deformities of the aorta and aortic valve, we believe, are caused by fibrosis of necrobiotic rheumatoid lesions.

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Derivation of Certain Forms of "Fibrinoid" from Smooth Muscle

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The term "fibrinoid"[1] has been applied to certain histologic alterations occurring within connective tissues and blood vessels.[2] Within small arteries and arterioles "fibrinoid" may be finely granular, lumpy, and granular or hyaline in appearance. Tinctorially,[2-4] vascular "fibrinoid" is intensely eosinophilic, intensely red with Masson's trichrome or Mallory's aniline blue stain, yellow with the Van Gieson stain, brown and black with silver stains, bluish-black with Weigert's fibrin stain, blue (orthochromatic) or metachromatic with toluidine blue, red-purple with the periodic acid-Schiff procedure, and purple or orange and purple with the phosphotungstic-acid-hematoxylin stain.

"Fibrinoid" has been described within blood vessels in a variety of disturbances, including malignant hypertension,[5] diabetes mellitus,[3] eclampsia,[6,7] periarteritis nodosa and other arteritides,[8,9] the generalized Shwartzman phenomenon,[10] experimental hypertensive cardiovascular states,[11-13] and thrombotic thrombocytopenia.[4,14]

Utilizing mainly frozen sections of formalin-fixed material, vascular "fibrinoid" has been observed to yield positive results with the following histochemical procedures: oil red O, Nile blue sulfate, Sudan black B, the Schultz reaction, the periodic acid-Schiff reagent, free potassium, free carbonyl groups, Congo red, and the protein-bound sulfhydryl procedure. These results suggest the presence of a multiplicity of ingredients within the "fibrinoid" of blood vessels—such as triglycerides and fatty acids; phosphatides, cholesterol, and cholesterol esters; aldehyde groups; sulfuric acid esters of polysaccharide origin; free potassium, and protein-bound sulfhydryl groups. Results such as these have been obtained with the vascular "fibrinoid" encountered in malignant hypertension of man,[15] experimental hypertensive cardiovascular states,[15] the generalized Shwartzman reaction,[16] and the kidney of diabetics.[17]

The "fibrinoid" change appears to depict a fundamental reaction to injury within the body. Yet the origin and nature of this material remain obscure.

Altshuler and Angevine[2] proposed the precipitation of the acid mucopolysaccharide of the ground substance of connective tissue as the common feature of "fibrinoid" formation. In some instances the precipitant was interpreted by these authors as an alkaline protein derived from necrosis of tissue. Schurmann and MacMahan[18] considered the passage of plasma and other ingredients of blood into the arteriolar wall as a basic feature in the development of arteriolar necrosis of malignant hypertension. Koss[3] accepted the "fibrinoid" within the kidney of diabetics as originating by the permeation of various structures by protein from the plasma. Masson, Corcoran, and Page[13] described the deposition of mucopolysaccharide in the arteriolar wall as the earliest alteration leading to the "fibrinoid" change that was observed in certain forms of experimental hypertension of the rat. Brunson, Thomas, and Gamble[19] studied the "fibrinoid" of the generalized Shwartzman

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reaction and suggested that some change in the circulating blood is involved in its deposition. Experimental observations concerning fibrinogen were presented by these workers in support of the hypothesis that "fibrinoid" is derived from the circulating blood and that fibrinogen may play a role in its formation.

Studies of the hypertensive cardiovascular state of the dog following bilateral nephrectomy and ureteral ligation in this laboratory [11,15,19-21] have led to the interpretation that the "fibrinoid" change observed in these preparations is derived mainly from altered smooth muscle of the media of the vessels involved. This autochthonous interpretation of the genesis of vascular "fibrinoid" has resulted from observations as follows: (1) the location of the "fibrinoid" within the area of the media and its precise continuity with normal or slightly altered media; (2) "fibrinoid" changes within individual smooth muscle fibers of the media and transitions between these isolated changes and the ultimate diffuse "fibrinoid" change; (3) similar staining, histochemical, and microspectroscopic characteristics of normal vascular smooth muscle, necrotic smooth muscle showing the "fibrinoid" change, and the hyalin of hyaline arteriolosclerosis when the frozen-section technique is used.[15,22,23] Related observations have been made with the vascular "fibrinoid" of malignant hypertension,[15] the diabetic's kidney,[17] the kidney in the Shwartzman reaction,[16] and an experimental lesion resembling that of thrombotic thrombocytopenia.[14]

Skelton [12] has reconsidered the problem of the origin of vascular "fibrinoid," utilizing the vascular lesions in rats treated with methylandrosterediol and desoxycorticosterone acetate. A combined interpretation of vascular "fibrinoid" was preferred, including the intramural transudation of protein and an autochthonous degeneration of smooth muscle.

"Fibrinoid" is identified by its tinctorial and histochemical characteristics.[3-11] Since the role of smooth muscle in the evolution of vascular "fibrinoid" remains obscure, it was considered of interest to ascertain whether normal smooth muscle autolyzed in the test tube can assume the characteristics of "fibrinoid," and whether this same material injected intravascularly would flow within the blood stream and lodge at various capillary sites. The kidney was chosen for this purpose, since "fibrinoid" has been described within it under different conditions. The results of such a study have been compared with observations made on the kidney in "malignant" hypertension of man.

Methods

Under sterile operating room conditions, smooth muscle was obtained from the muscularis of the stomach and colon of the dog. One operator with sterile gloves and sterile equipment made the abdominal incision, and another operator collected the specimens. Perforation into the lumen was prevented by careful manipulation. The shavings of smooth muscle were placed in sterile test tubes. The muscle was allowed to undergo autolysis at room temperature for 12 to 24 hours. The mass of muscle was then subjected to the action of a Waring Blendor under sterile conditions, as follows: About 5 gm. of autolyzed muscle and 250 cc. of sterile isotonic saline were acted on by the Blendor for 15 minutes, allowed to cool, and subjected to the action of the Blendor for an additional 15 minutes. The resultant material was allowed to stratify, and the supernatant was removed.

Characterization of Autolyzed Smooth Muscle.—The mass of autolyzed and macerated muscle was subjected to staining and histochemical procedures by three different approaches. In one approach the amorphous material was smeared on slides and then subjected to the various procedures. In another, the material was mixed with warm absorbable gelatin sponge U. S. P. (Gelfoam) and allowed to solidify into a button. This button was sectioned by the frozen-section technique, then subjected to the procedures. In the third approach the staining and histochemical procedures were conducted on the gross material in the test tube. About 3 ml. of the mass was used for the latter procedure.

The autolyzed muscle was then subjected to the following staining and histochemical procedures, as specified by the references given: (1) hematoxylin and eosin stain [24]; (2) Mallory's aniline blue stain [25]; (3) Masson's trichrome stain [26]; (4) Van Gieson stain [27]; (5) phosphotungstic acid-

hematoxylin stain[25]; (6) oil red O[26]; (7) Nile blue sulfate[26]; (8) Sudan black B[26]; (9) periodic acid-Schiff reagent[27]; (10) potassium[28]; (11) free carbonyl[28]; (12) protein-bound sulfhydryl[28], and (13) Congo red.[29]

The histochemical procedures (Procedures 6-12) yielded the same results with isotonic saline prepared with local distilled water, which is slightly acid and with an M/10 phosphate buffer of pH 7.4. The staining procedures (Procedures 2-5) yielded results with unbuffered saline which differed from those with the phosphate buffer. These differences will be described in the section on results.

Injection of Autolyzed Smooth Muscle.—Smooth muscle, removed from the stomach and/or colon of the dog under sterile conditions, as described above, was macerated in a Waring Blendor, then allowed to autolyze for about 12 hours at room temperature while suspended in M/10 phosphate buffer of pH 7.4. The desired particle size was obtained by filtering the sediment through gauze of diminishing porosity until the suspension could be made to pass easily through a 25-gauge needle. All manipulations were conducted with sterile equipment and with sterile precautions.

Approximately 40 gm. of smooth muscle was suspended in 1000 ml. of the buffer solution and then filtered. The exact quantity of muscle in the final suspension used was not known. The particle size varied considerably from experiment to experiment.

Mongrel dogs, apparently normal, weighing between 11 and 16 kg. were used. The animal was anesthetized with sodium pentobarbital given intravenously. Through a flank incision the kidney was delivered and the renal pedicle was isolated and clamped. The renal artery was isolated, temporarily clamped, and with a 23- or 24-gauge needle 30 ml. of the suspension of smooth muscle was injected into the renal side of the artery before its bifurcation. After injection the pedicle was released. The kidney was returned to its bed, and the incision was closed.

The kidney was removed 3 to 24 hours after the injection. It was fixed in 10% neutral formalin. The following stains were conducted on sections obtained from paraffin blocks: hematoxylin and eosin, Mallory's aniline blue, Masson's trichrome stain, Van Gieson's method, the Gram-Weigert stain,[27] Wilder's silver stain,[28] toluidine blue,[26] periodic acid-Schiff reagent, and phosphotungstic acid-hematoxylin. Histochemical procedures were performed on frozen sections with oil red O, Nile blue sulfate, Sudan black B, the periodic acid-Schiff (PAS) reagent, free potassium, free carbonyl groups, Congo red, and protein-bound sulfhydryl.

Seventeen experiments (the injection of one kidney of 17 dogs) were conducted. The kidney was recovered after 12 to 24 hours in 11 experiments and after 3 to 4 hours in the remainder. The opposite kidney served as the normal control.

One additional experiment was performed, using finely divided, autolyzed muscle which had been colored in the test tube with Sudan black B and PAS, as described above. Muscle colored black with Sudan black B was used in one kidney, and material colored red-purple with the periodic acid-Schiff reagent was used in the other kidney. After three hours the kidneys were removed, fixed in formalin, sectioned while frozen, and studied without the addition of other stains.

The Glomerulus in Malignant Hypertension.—A third study consisted of the identification of "fibrinoid" material within capillaries of glomeruli of persons with "malignant" hypertension, utilizing the same staining and histochemical techniques listed above. The possible source of this glomerular material from the afferent arteriole was considered. For this purpose renal tissue obtained at necropsy and fixed in 10% neutral formalin was used.

The kidneys were obtained from subjects displaying a prominent hypertension clinically and arteriolar necrosis microscopically. The general characteristics of the cases used are given in Table 1.

The hematoxylin-eosin, Mallory aniline blue, Masson trichrome, and Van Gieson stains were performed in conventional fashion on material prepared by paraffin block. Paraffin block was also used for the Wilder silver stain and the Gram-Weigert fibrin stain. The periodic acid-Schiff procedure was conducted on both frozen and paraffin sections, while the Congo red procedure was confined to frozen-section material. The Nile blue sulfate, oil red O, and Sudan black B stains were performed on frozen sections of formalin-fixed material. The toluidine blue stain was conducted on frozen sections at pH 4.5. The potassium and protein-bound sulfhydryl procedures were performed on frozen sections, while the free-carbonyl procedure was carried out on paraffin sections.

Results

I. *Derivation of "Fibrinoid" from Smooth Muscle by Autolysis in the Test Tube.*—Microscopically, the autolyzed muscle appeared either as a finely granular, amorphous material or as small bundles within which altered fibers could be identified. The appearance of the substance of these identifiable fibers was the same as that of the amorphous material. In some fibers a shriveled or pyknotic nucleus was present.

TABLE 1.—*Main Characteristics of Cases Used in Present Study*

Case	Age	Race	Sex	Diagnosis*	B.P.	BUN	Kidney at Autopsy
1	44	C	M	Scleroderma; renal failure	190/100		Acute and chronic pyelonephritis
2	80	W	F	Cerebrovascular accident and coma	120/70		Acute arteriolar necrosis; thecoma of ovary
3	75	C	M	Pyelonephritis; renal failure	180/120	213	Acute and chronic pyelonephritis
4	82	W	M	HCVD and renal failure	200/120	200	Chronic pyelonephritis
5	51	C	M	HCVD; lobar pneumonia	196/120		Chronic pyelonephritis
6	73	W	F	Eclampsia; HCVD	200/160		Bilateral cortical necrosis and chronic pyelonephritis
7	50	C	M	HCVD and renal failure	180/120	194	Chronic pyelonephritis
8	46	C	M	HCVD	260/150		Acute pyelonephritis; secondary periarteritis nodosa
9	47	C	M	HCVD	220/140	132	Chronic pyelonephritis
10	38	W	M	HCVD; cerebral infarction	260/140		Chronic glomerular nephritis
11	63	W	M	HCVD and renal failure	250/120	126	Benign and malignant arteriolar nephrosclerosis
12	42	W	M	HCVD; subarachnoid hemorrhage	210/130	36	Acute and chronic pyelonephritis
13	81	W	F	Hematuria; melena			Acute and chronic pyelonephritis; necrotizing papillitis

* HCVD indicates hypertensive cardiovascular disease apparently of the essential type.

The appearance and the staining and histochemical reactions were those of vascular "fibrinoid" as encountered in different conditions, such as malignant hypertension of man, comparable experimental states, the kidney of diabetics, and the kidney in the generalized Shwartzman reaction. Thus the autolyzed muscle was intensely eosinophilic, red with Mallory's aniline blue and Masson's trichrome stain, yellow with the Van Gieson stain, blue with toluidine blue, purple with the phosphotungstic acid-hematoxylin stain, blue-black with Weigert's fibrin stain, brownish black with the silver stain, red-purple with the periodic acid-Schiff reagent, positive (orange color) with the Congo red stain, positive (black) with the free-potassium procedure, positive (purple) with the free-carbonyl procedure, positive (reddish brown) with the protein-bound sulfhydryl procedure, markedly positive (red) with oil red O, markedly positive (black) with Sudan black B, and markedly positive (deep blue) with Nile blue sulfate (Fig. 1).

Where bundles of fibers could be identified, the membrane about each fiber stained as a fine structure and at times in a manner different from that of the main body of the fiber. Thus, with the aniline blue stain this membrane at times stained faintly blue; with the trichrome method it stained faintly green, and with the phosphotungstic acid-hematoxylin stain it stained orange.

The above responses to the histochemical procedures were the same regardless of whether the saline used was slightly acid or basic. When the saline used with the muscle was acid, the results with the staining procedures differed from those when M/10 phosphate buffer pH 7.4 was used. This was well demonstrated with the gross staining of the amorphous mass. Thus, with the acid medium the mass was pink with the Van Gieson stain, blue with Mallory's aniline blue stain, orange with the phosphotungstic acid-hematoxylin stain, and green with Masson's trichrome stain. When the alkaline buffer was used, the same material became yellowish pink with the Van Gieson stain, red with Mallory's aniline blue and Masson's trichrome stain, and purple with the phosphotungstic acid-hematoxylin stain. The material prepared grossly when smeared on slides had the same microscopic appearance as the material prepared after smearing or after being enmeshed in absorbable gelatin sponge U. S. P.

II. *Reproduction of Embolic Vascular "Fibrinoid" by Injection of Autolyzed Smooth Muscle into Renal Artery of Dog.*—In 12 to 24 hours after the injection of the finely divided, autolyzed smooth muscle into

Fig. 1.—The gross appearance of amorphous, granular, autolyzed smooth muscle following various histochemcial and staining procedures, as specified, is depicted. *SH* stands for the protein-bound sulfhydryl procedure; *PAS* stands for the periodic acid-Schiff procedure; *PTAH* stands for the phosphotungstic acid-hematoxylin stain, and *keto* stands for the free carbonyl procedure. The other labels are self-explanatory. The colors were originally interpreted as given in the text.

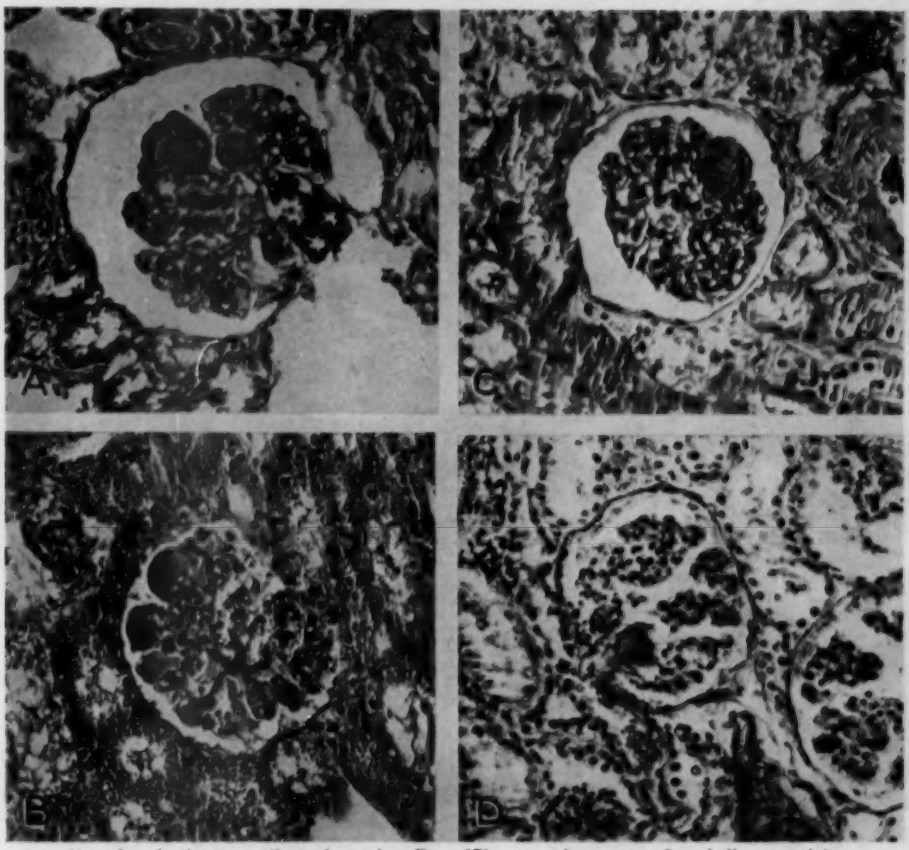

Fig. 2.—*A* (hematoxylin-eosin stain; Dog 17), several masses of embolic material are pictured at the periphery of the glomerulus. The larger mass is finely vacuolated. The other masses are round and irregularly elongated. The material, derived from autolyzed normal smooth muscle, has the characteristics of "fibrinoid" and is identical with the material encountered in the glomerulus in "malignant" hypertension and diabetes in man and in the Shwartzman reaction in the rabbit.

B (hematoxylin-eosin stain; Dog 16), in this section the embolic masses in the glomerulus tend to be round in cross section and are hyaline in appearance. The endothelial cells of the glomerular capillaries are closely applied to the emboli of autolyzed smooth muscle.

C (hematoxylin-eosin stain; Dog 17), the roundish embolus of this section has the appearance of a so-called "intercapillary lesion." It is embolic in origin and consequently should be within a prominently distended capillary.

D (Fortis silver stain; Dog 17), two embolic masses within a glomerulus are shown. The one at the hilus has wavy fibrillae of silver-positive material. Otherwise the material took a light-brown stain.

Illustrations reduced to about 65% of mag. × 365.

the renal artery the kidney usually revealed multiple infarcts. The infarction appeared to result mainly from the obstruction of interlobular arteries with the injected material. The kidneys removed within three to four hours following the injection revealed mainly the occlusive masses within various vessels.

The occlusive material was found within interlobular arteries and glomerular arterioles and in the glomerular capillaries (Figs. 2-7). The material was eosinophilic and appeared finely granular, lumpy, and granular or hyaline, thus reduplicating the appearance of the vascular "fibrinoid" as encountered in a variety of conditions.[3-17]

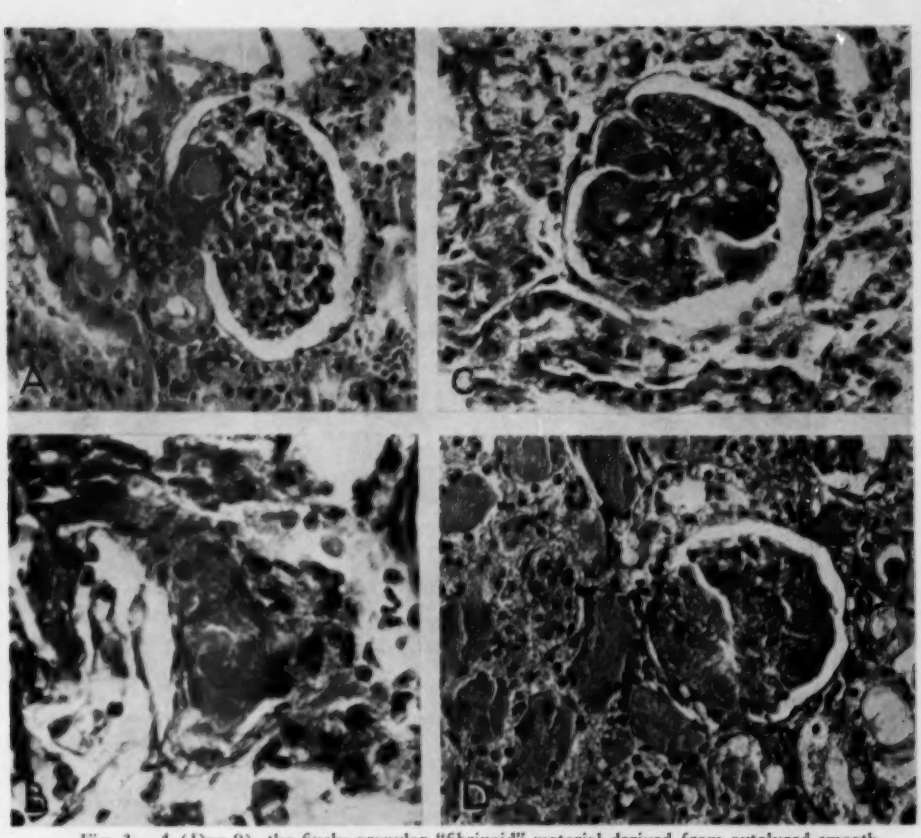

Fig. 3.—*A* (Dog 9), the finely granular "fibrinoid" material derived from autolyzed smooth muscle apparently lodged in and impacted the afferent arteriole at the hilus of the glomerulus. The glomerulus appears collapsed and almost without red blood cells. Hematoxylin-eosin stain; reduced to 68% of mag. × 365.

B (Dog 16), an elongated embolic mass partly in the afferent arteriole and partly within the glomerulus is shown. This type of continuity has been observed in cases of "malignant" hypertension of man. Hematoxylin-eosin stain; reduced to 68% of mag. × 700.

C (Dog 8), this glomerulus contains several masses of "fibrinoid" (embolic autolyzed smooth muscle), but, in addition, a prominent glob of this material is observed free in Bowman's space. This type of escape allows the material to gain access to the tubular lumina and become incorporated in tubular casts. Hematoxylin-eosin stain; reduced to 68% of mag. × 400.

D (Dog 8), this glomerulus depicts infarction and granular dissolution of its structure. Within such an altered glomerulus the "fibrinoid" can be identified. A similar change is observed in "malignant" hypertension in man. Hematoxylin-eosin stain; reduced to 68% of mag. × 365.

Within most sites the material revealed a fairly homogeneous appearance. In an occasional larger artery remnants of smooth muscle nuclei could be seen within the obturating mass (Figs. 4 and 5). In some examples the injected material distended the afferent arteriole at its entrance into the glomerulus (Fig. 3*A* and *B*). With the latter condition the glomerulus was either collapsed or infarcted. Within the collapsed glomerulus the cells were closely arranged, yielding a false impression of hypercellularity.

The glomerular capillaries were frequently distended with the embolic masses (Figs. 2 and 3). In this location the "fibrinoid" assumed a more hyaline appearance. The occlusive masses in the glomerular capillaries

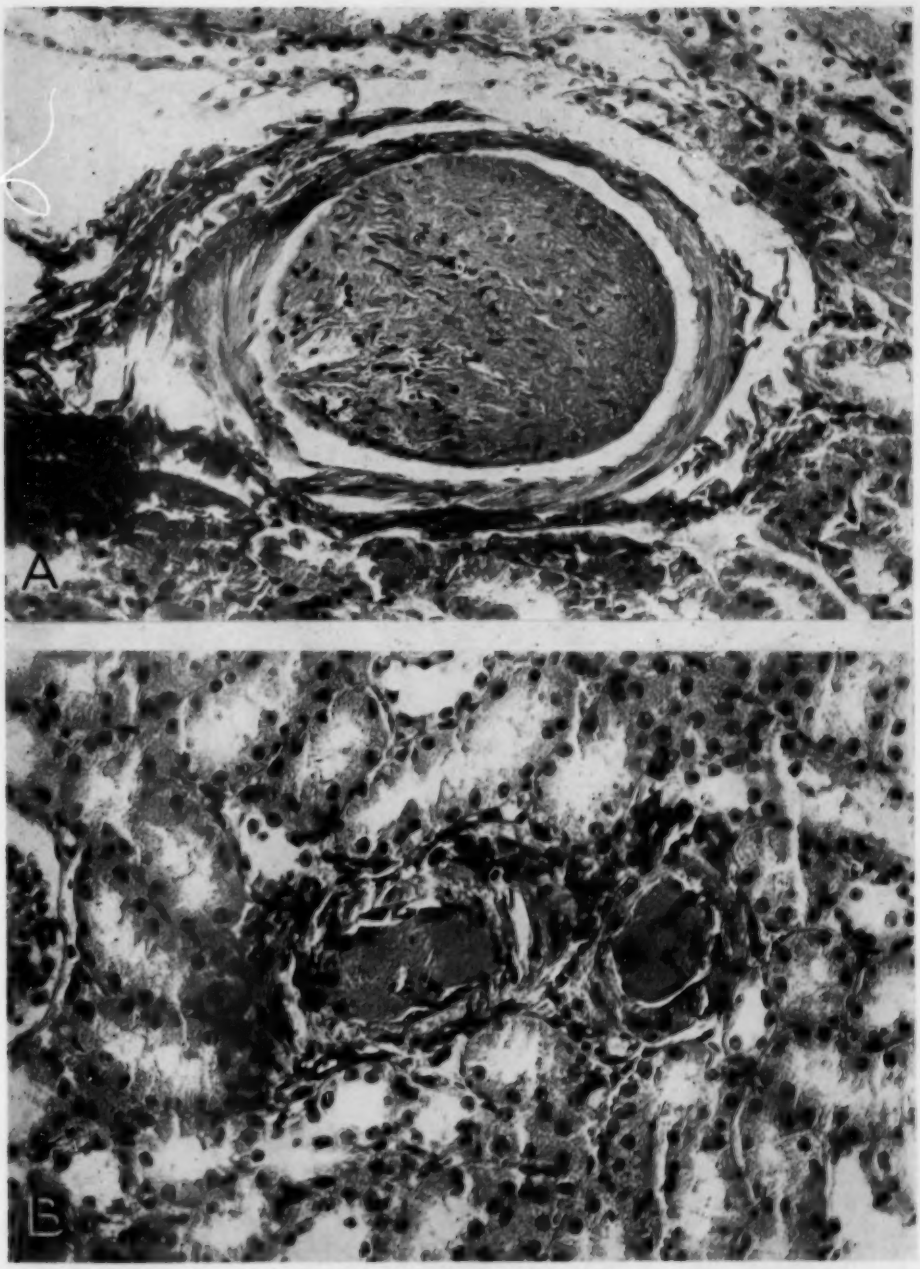

Fig. 4.—*A* (Dog 4), this artery within the kidney is occluded by a mass of partly autolyzed muscle. The nuclear pattern of the muscle can still be identified, although the nuclei are mainly pyknotic. The cytoplasmic substance of the fibers has become partly fused and very finely granular. This fused portion, even at this stage, yields the staining and histochemical characteristics of vascular "fibrinoid." Hematoxylin-eosin stain; reduced to 87% of mag. × 310.

B (Dog 5), these segments of an interlobular artery are occluded with the embolic masses. The wall of the artery shows signs of injury (degeneration and necrosis of the media) and infiltration with inflammatory cells (polymorphonuclear neutrophilic leukocytes and lymphocytes). This is a form of embolic occlusion and acute arteritis which has been observed in other embolic phenomena. Hematoxylin-eosin stain; reduced to 87% of mag. × 365.

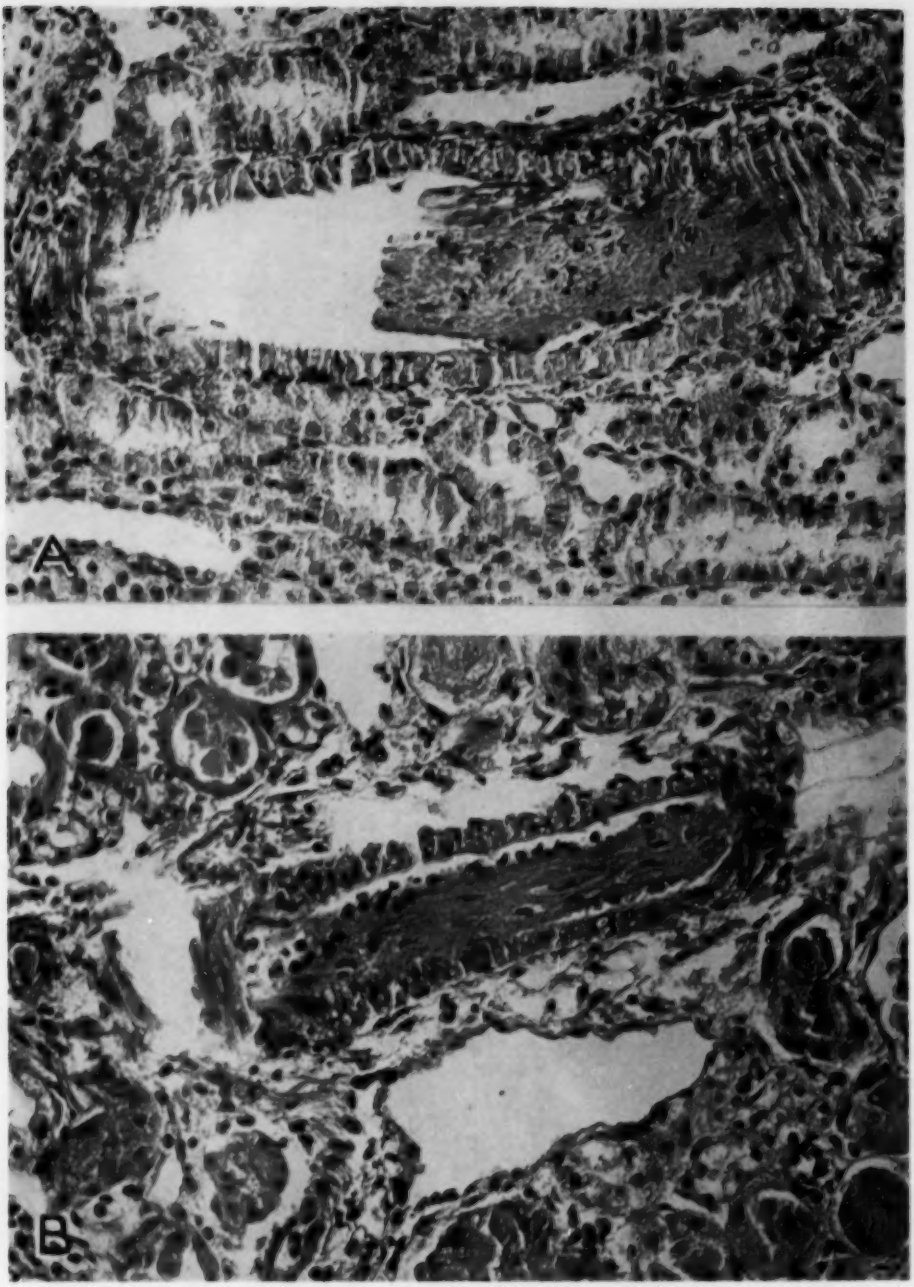

Fig. 5.—*A* (Dog 9), this interlobular artery is partly occluded with the mass of altered smooth muscle. The latter seems to be attached to the endothelial surface and is partly infiltrated by leukocytes. Of pertinent interest is the alteration of the media of this artery. Individual smooth muscle fibers are degenerated and necrotic in a "block-like" fashion. These individual units of altered smooth muscle have the same staining and histochemical characteristics as the occlusive mass within the lumen.

B (Dog 8), another example similar to that of *A* above. The nuclei of the smooth muscle fibers can still be identified within the occlusive mass. There is a "block-like" arrangement of altered smooth muscle fibers in the media, and in some areas these altered fibers appear to be fusing into a more diffuse "fibrinoid" change. Hematoxylin-eosin stain; reduced to 87% of mag. × 365.

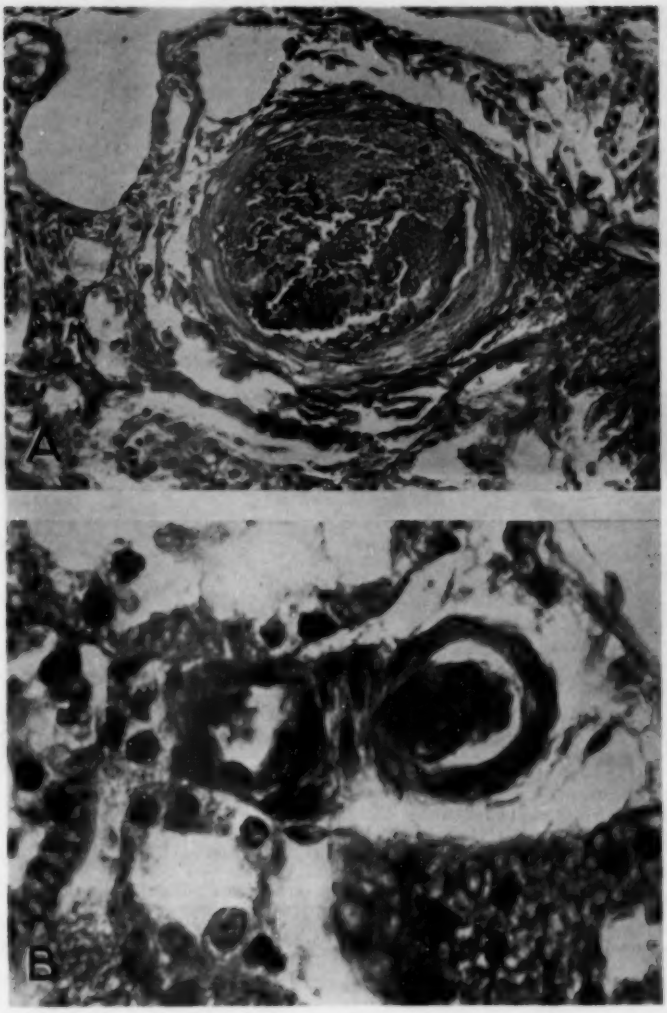

Fig. 6.—*A* (Dog 17), this occlusive mass within an interlobular artery is prominently permeated by neutrophilic leukocytes. Hematoxylin-eosin stain; reduced to 80% of mag. × 310.

B (Dog 4), this small interlobular artery is occluded with the embolic material derived from smooth muscle. This material appears to be closely adherent to the wall. It has a lumpy and granular appearance. Hematoxylin-eosin stain; reduced to 80% of mag. × 900.

had different shapes, such as round, oval, and elongated. Globs of the material were encountered in the capsular space, indicating an escape from within the glomerular capillaries (Fig. 3*C*).

Not only did the embolic masses resemble vascular "fibrinoid" with the hematoxylin-eosin stain but they also displayed other tinctorial and histochemical properties of this type of "fibrinoid." Thus they were intensely red with Masson's trichrome stain and Mallory's aniline blue stain, yellow with Van Gieson's stain, dark blue with the Gram-Weigert stain, brown with black fibrils with Wilder's silver stain (Fig. 2*D*), blue or slightly metachromatic with toluidine blue, and orange or orange and purple with the phosphotungstic acid-hematoxylin stain. The PAS procedure gave negative or weakly positive results when paraffin-block technique was used. With frozen sections the following procedures gave markedly positive results: oil red O (red), Nile blue sulfate (mainly blue), Sudan black B (black), periodic acid-Schiff reagent (red-purple), free potassium (black), free carbonyl groups

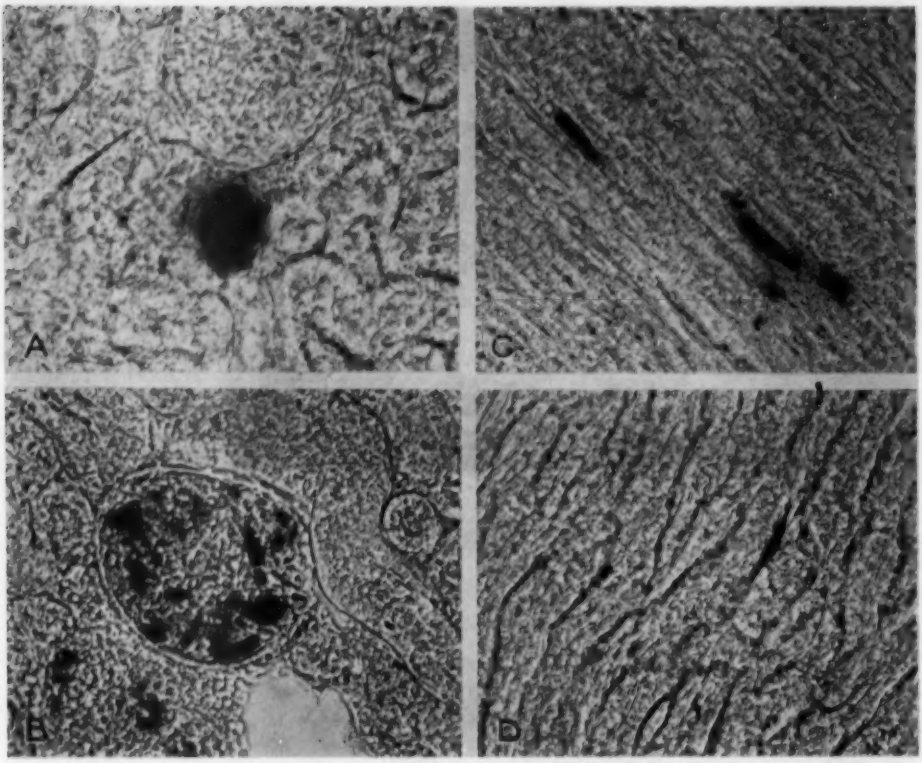

Fig. 7.—*A* (PAS procedure), the finely divided, autolyzed muscle was colored red-purple with the periodic acid-Schiff procedure, then injected into the renal artery. The kidney was sectioned, frozen, and observed and photographed unstained. The material is shown in an interlobular artery near a glomerulus. It is intensely PAS-positive. *B* (Sudan black B), in this example the autolyzed muscle was colored black with Sudan black B and then injected into the renal artery. The kidney was sectioned, frozen, and observed without an additional stain. The Sudan black B-positive material is observed within glomerular capillaries. The same appearance has been obtained when the particles of muscle are injected unstained and the section is treated with Sudan black B subsequently.

C (Sudan black B), another section from the kidney of Figure 6*B*. The Sudan black B-positive material is seen as casts within straight tubules in the medulla. This observation supports the escape of the material through glomerular capillaries and its travel down the tubule.

D (Sudan black B), the same kidney as that in Figure 6*B* and *C*. The Sudan black B-positive material is observed in the peritubular area. This finding is interpreted as resulting from the passage through the glomerular capillaries, followed by impaction within the peritubular capillaries.

All illustrations reduced to about 52% of mag. ×310.

(purple), Congo red (orange), and protein-bound sulfhydryl (reddish brown). Thus, after embolization within the body the material continued to display the tinctorial and histochemical characteristics of smooth muscle autolyzed in the test tube and of the "fibrinoid" of small arteries and arterioles encountered in malignant hypertension, diabetes, the general Shwartzman reaction, experimental hypertensive cardiovascular states, and thrombotic thrombocytopenia.

Isolated groups of tubules contained fine, round globules of material having the same characteristics as the masses in the glomeruli. In the medulla, casts of the material were observed within tubules (Fig. 7*C*). The presence of the injected material in the

peritubular area, apparently within peritubular capillaries, was also observed (Fig. 7*D*).

Clusters of polymorphonuclear neutrophilic leukocytes were, on occasions, observed about the masses of autolyzed smooth muscle within arteries, arterioles, and capillaries (Figs. 5*A* and 6*A*).

Another observation of interest consisted of necrosis of the media of certain interlobular arteries which were distended with an occlusive mass of autolyzed muscle (Figs. 4*B* and 5*A* and *B*). The necrosis occurred by itself or in conjunction with the infiltration of the arterial wall and adventitia by neutrophilic leukocytes (Fig. 4*B*). The latter combination had the characteristics of an arteritis. Necrosis of the media with or without inflammation has been observed in pulmonary arteries following embolic occlusions experimentally induced.[40]

Although the specific mechanism of the above type of vascular necrosis is not known, it is of interest that the necrotic smooth muscle of the media of such arteries assumed the characteristics of "fibrinoid," thus having the same characteristics as the embolic occlusive mass.

III. *The "Fibrinoid" in the Glomerulus in Malignant Hypertension.*—The "fibrinoid" material within the necrotic glomerular arteriole and within the glomerulus had the same appearance and identical staining and histochemical properties. These are tabulated in Table 2.

Within the glomerulus the "fibrinoid" either was in direct continuity with that within the arteriole (Figs. 8*B* and 9*A*) or was present as separate and distinct masses within one or more glomerular lobules (Figs. 8*A* and 9*B*). The separate masses assumed several shapes, such as oval, round, and elongated (Fig. 8*A* and *B*). On rare occasions the masses appeared to have a small central lumen, thus giving the appearance of a thickened capillary wall.

The staining characteristics of the "fibrinoid" material were those repeatedly alluded to in the literature.[2-4] The histochemical results indicated the presence of a multiplicity of ingredients, similar to those observed in other states which display vascular "fibrinoid."[15-17]

The casts in some tubules yielded the same staining and histochemical properties as the arteriolar and glomerular "fibrinoid," while casts in other tubules did not reflect these features.

Comment

The results herein recorded indicate that the staining and histochemical characteristics of smooth muscle autolyzed in the test tube are the same as those of the "fibrinoid" observed in the wall of small arteries, arterioles, and capillaries in a variety of conditions, including malignant hypertension of man, experimental hypertensive cardiovascular states, the kidneys of diabetic subjects, the generalized Shwartzman reaction, and

TABLE 2.—*Staining and Histochemical Characteristics of "Fibrinoid" Within the Glomeruli and Afferent Arterioles in Malignant Nephrosclerosis**

Stain and Histochemical Procedure	Necrotic Arteriole	Glomerular Fibrinoid
Hematoxylin and eosin	Eosinophilic	Eosinophilic
Mallory's aniline blue	Intensely red	Intensely red
Trichrome (Masson)	Intensely red	Intensely red
Van Gieson	Yellow	Yellow
Silver (Wilder)	Light gray-brown	Light gray-brown
Gram-Weigert	Dark purple	Dark purple
PAS	Red-purple	Red-purple
Toluidine blue	Blue (orthochromatic)	Blue (orthochromatic)
Oil red O	Red	Red
Nile blue sulfate	Blue	Blue
Sudan black B	Black	Black
Free carbonyl K	Purple Black	Purple Black
Protein-SH	Reddish brown	Reddish brown
Congo red	Orange	Orange

* The two structures yield "fibrinoid" with the same characteristics.

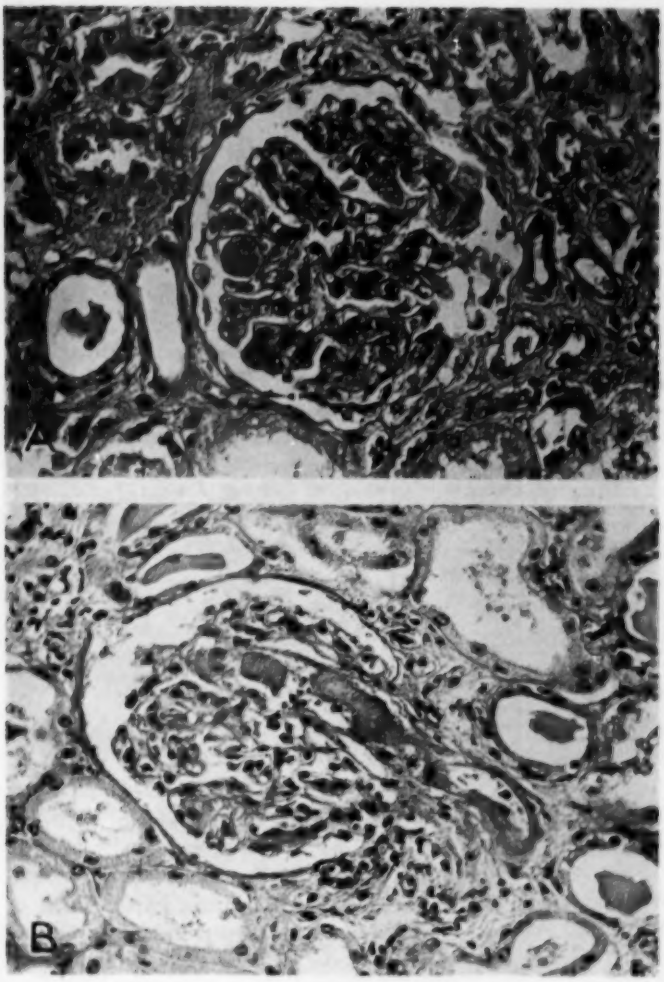

Fig. 8.—Malignant hypertension in man. *A*, this glomerulus contains a round mass of eosinophilic material in its periphery. The material is finely vacuolated. This type of material has the staining and histochemical characteristics of the necrotic arteriole and of smooth muscle autolyzed in the test tube. We interpret this round mass as being within a glomerular capillary loop. Hematoxylin-eosin stain; reduced to 2/3 of mag. × 350.

B, the glomerular arteriole, apparently afferent, is necrotic and has the characteristics of "fibrinoid." This "fibrinoid" material is continuous with similar material within the glomerulus. The glomerular material also has the characteristics of "fibrinoid." We interpret the glomerular "fibrinoid" as being within a glomerular capillary loop and as being derived from the material in the arteriolar wall, which we consider to consist mainly of necrotic smooth muscle of the media of the arteriole. Hematoxylin-eosin stain; reduced to 2/3 of mag. × 320.

certain experimental lesions resembling those of thrombotic thrombocytopenia.

Smooth muscle, autolyzed in the test tube, can be divided into fine particles which flow readily through a 24-gauge needle. When these particles are injected into the renal artery of the dog, the embolic masses pass along and lodge within interlobular arteries, arterioles, glomerular capillaries, and peritubular capillaries. Within these locations the autolyzed smooth muscle continues to exhibit the staining and histochemical characteristics of vascular "fibrinoid," as observed in a variety of conditions. The embolic masses in glomerular capillaries may break through into Bowman's space, pass down the tubule, and become incorporated in casts.

The lodging of emboli of autolyzed smooth muscle within interlobular arteries may be associated with necrosis of the smooth muscle of the media of the artery. Under these conditions the necrotic vascular smooth muscle assumes the same characteristics as the occlusive embolic mass, and these are the characteristics of vascular "fi-

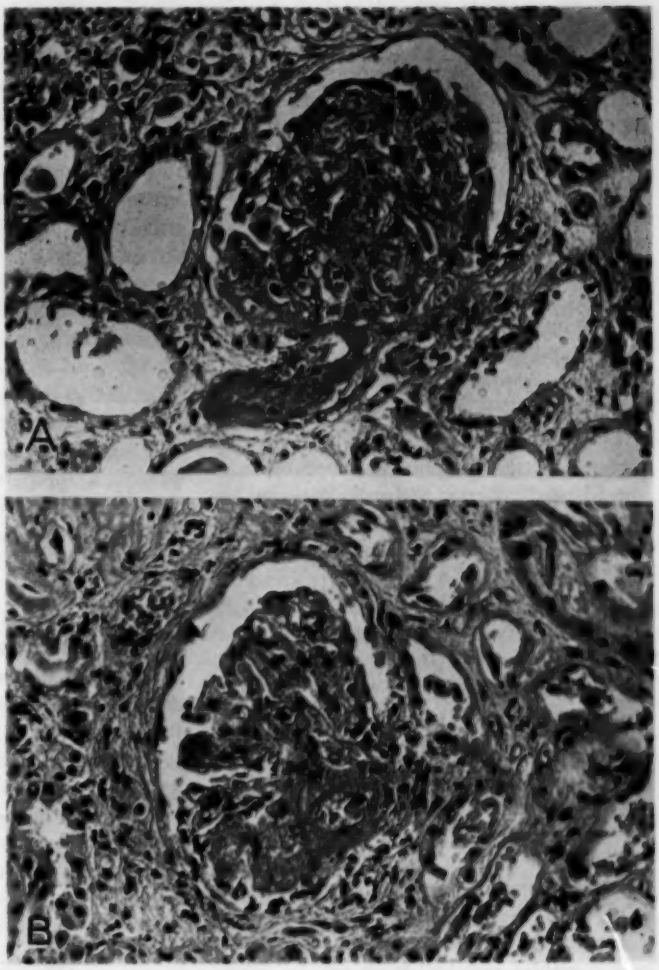

Fig. 9.—Malignant hypertension in man. *A*, the "afferent" arteriole is collapsed and replaced by "fibrinoid," which we interpret as derived mainly from the necrotic media. This material is continuous with similar material within the glomerulus. At the same time, most of the glomerulus has become disintegrated and fused into an amorphous structure.

B, the arteriole near the hilus of the glomerulus is necrotic and its wall consists of "fibrinoid." The lower lobule of this glomerulus is necrotic and of a smudged appearance. We consider this lobular change to result from embolization by "fibrinoid" or necrotic smooth muscle, followed by infarction and disintegration.

Hematoxylin-eosin stain; reduced to 2/3 of mag. × 350.

brinoid." This arterial change may or may not be associated with a superimposed acute arteritis.

The deposition of "fibrinoid" within the glomeruli in cases of "malignant" hypertension herein considered was sparse and did not appear to represent a widespread complication. Thus, the deposition was minimal when compared with that occurring in the kidney of certain diabetic subjects and in the generalized Shwartzman reaction. It may have represented a terminal or near-terminal complication, and certainly its meaning from the view of altered renal function or abnormal composition of the urine is not known. This deposition, on the other hand, as a source of further consideration of vascular "fibrinoid" and its possible origin may have fundamental importance.

The staining and histochemical characteristics of the glomerular "fibrinoid" in malignant hypertension are the same as those observed in a group of conditions in which vascular "fibrinoid" occurs, and of normal smooth muscle which is allowed to undergo autolysis in the test tube.

In certain preparations, such as the nephrectomized dog, [11,15,19-21] transitions be-

tween normal smooth muscle fibers, individually altered fibers, and the ultimate "fibrinoid" changes have been described. Moreover, in all of these states the "fibrinoid" change is particularly outstanding within the area of the media, where it is frequently in direct continuity with normal appearing smooth muscle. These are among the major features which have been used in support of the view that vascular "fibrinoid" is derived mainly from degradation of smooth muscle of the media of small arteries and arterioles.[11,15,19-22]

The migration of the altered smooth muscle as "fibrinoid" into the arterial and arteriolar lumen has been proposed as a consequence of the morphologic appearance of certain small arteries and arterioles and the presence of occlusive masses of similar appearance within these vessels and nearby capillaries.[11,14,16,17] A similar hypothesis for the deposition of "fibrinoid" within the glomeruli in "malignant" hypertension appears attractive. This view is based on the similarity between the arteriolar "fibrinoid" and the "fibrinoid" within the nearby glomeruli by staining and histochemical methods, the proximity of the two structures, the morphologic suggestion of the migration of the arteriolar "fibrinoid" into the arteriolar lumen, the morphologic demonstration of an apparent streaming of the arteriolar "fibrinoid" into the glomerular vessels at the glomerular hilus, and the presence of "fibrinoid" within certain renal tubules. The presence of "fibrinoid" in certain tubular casts may be considered in keeping with the periodic escape of "fibrinoid" through the glomerular capillaries and into Bowman's space and the tubule.

We present these various observations as collateral support for the view that the "fibrinoid" of small arteries, arterioles, and capillaries observed in a variety of conditions is derived mainly from altered smooth muscle of the media. This concept places emphasis on the need for greater understanding of the mechanisms causing injury of smooth muscle in these various conditions.

Summary and Conclusions

Normal smooth muscle allowed to undergo autolysis in the test tube and then macerated develops the same staining and histochemical properties of the "fibrinoid" as those observed in the small arteries, arterioles, and capillaries of a variety of conditions, including malignant hypertension, comparable experimental states, the kidney of diabetic subjects, the Shwartzman reaction, and thrombotic thrombocytopenia. The color developed by the altered muscle with certain stains (Mallory's aniline blue and phosphotungstic acid-hematoxylin, Masson's trichrome stain, and the Van Gieson stain) appears to be dependent on the pH of the preparation.

Normal smooth muscle, autolyzed in the test tube and divided into small particles, will flow readily through a 24-gauge needle. The particles can be injected into the renal artery of the dog. Under these conditions the embolic masses pass along the renal vasculature and lodge in the interlobular arteries, arterioles, glomerular capillaries, and peritubular capillaries.

The embolic masses of autolyzed smooth muscle in the above locations have the staining and histochemical characteristics of vascular "fibrinoid" observed in a variety of conditions, including malignant hypertension, similar experimental states, the kidney in diabetes, the generalized Shwartzman reaction, and thrombotic thrombocytopenia.

The embolic masses in the glomerular capillaries may break through into Bowman's space and pass down the tubule. Here they may become incorporated in casts or may break up into fine particles.

The lodging of the emboli in the interlobular artery may be associated with necrosis of the smooth muscle of the media of the artery. Under these conditions the necrotic vascular smooth muscle assumes the same characteristics as the occulsive mass, namely, the characteristics of vascular "fibrinoid." This change may or may not be associated with a superimposed acute arteritis.

The "fibrinoid" within the glomeruli of kidneys from subjects with malignant hypertension displays the same staining and histochemical properties as the "fibrinoid" of the afferent glomerular arteriole which appears necrotic.

The "fibrinoid" of the arteriole and glomerulus in malignant hypertension seems to contain a multiplicity of ingredients, such as fats, fatty acids, phospholipids, aldehyde groups of polysaccharide origin, free potassium, free carbonyl groups, sulfuric ester of mucopolysaccharide origin, and protein-bound sulfhydryl groups.

The possibility of the origin of vascular "fibrinoid" from altered smooth muscle of the media has been previously entertained. The thesis that the glomerular fibrinoid of malignant hypertension is derived from the arteriolar fibrinoid by the process of embolization has been entertained in the present study.

The observations recorded are considered to add to the concept that vascular "fibrinoid" as observed in a variety of conditions is derived mainly from altered smooth muscle of the media.

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Infectious Pancreatic Necrosis in Trout

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Since the report of pancreatic necrosis in young fingerling brook trout, Salvelinus fontinalis, by Wood et al.,[1] an increasing number of outbreaks of this disease have been observed in different parts of the country. Some outbreaks occurred also among small fingerling rainbow trout (Salmo gairdneri). However, diagnosis in rainbow trout has been made only on the basis of gross symptoms, which are characterized as intermittent whirling or corkscrewing of the affected individuals, lack of food in the entire intestinal tract, and the presence of nearly colorless mucus in the more or less distended gut. The flow of bile appears almost completely stopped. Since M'Gonigle [2] did not include histopathological data in his report, there is no absolute certainty that the disease described by him is identical with pancreatic necrosis in trout. However, since the behavior of the diseased trout and the gross symptoms were exactly the same, it is likely that the diseases reported by M'Gonigle and by us are identical.

Histopathological examination of trout from the 1954 outbreak at the Leetown station revealed acute pancreatic necrosis. Necrotic changes in voluntary muscles of the affected fish collected from the outbreaks in 1955 have furnished additional facts, which are here presented, supporting the hypothesis that the agent of this disease may belong to the Coxsackie group of viruses.

Material and Experiments

In the winter of 1955 the disease again affected at the Leetown station fingerling brook trout that hatched simultaneously from two unrelated batches of eggs received from the same sources as those in 1954 (Berlin, N. H., and Bellefonte, Pa.). As in 1954, the disease started first in the Bellefonte trout and was then apparently transmitted to the Berlin group. In the winter of 1956 no trout were hatched from eggs received from the Bellefonte hatchery and no outbreak occurred. The association of pancreatic necrosis with eggs received from the same source may serve as additional evidence of the infectious nature of the disease and its transmission by fish eggs. It is possible that the infective agent survives inside the trout eggs, since the external disinfection of eggs with sodium-*p*-(ethylmercuri)thiophenylsulfonate (Sulfo-Merthiolate) did not prevent the outbreaks of the infection.

In the outbreak which occurred in 1955 the peak of mortalities occurred about Feb. 10 in the Bellefonte trout and about Feb. 28 in the Berlin trout. The highest mortality for a five-day period was 19% in both strains of trout. The total loss calculated from the hatchery mortality records was 62% for the Bellefonte trout and 77% for the Berlin trout. Since routine hatchery mortality records are not very accurate and losses from all other causes are lumped together, the difference between these two figures cannot be considered significant.

Reliable figures have been obtained, however, by observations carried out on especially selected samples of the Berlin brook trout which were transferred to experimental troughs just after the presence of the disease became apparent. Four groups were selected. Groups A and B were taken from troughs in which the disease was causing heavy mortality. In Group C the disease symptoms were just appearing, and Group D was believed free of the disease. The comparative mortality in the four groups is shown in Figure 7. Losses were the lowest in Group D, in which the disease was apparently in the incubating stage during the first 15 days of observation. This fact may serve as support for the observation reported by M'Gonigle [2] that mortalities caused by this disease decrease with the increasing age of the affected trout. It indicates, too, an additional similarity between this condition and the Coxsackie virus group, which is also much more pathogenic to young mice and hamsters,[3-5] with a decreasing pathogenicity as the host's age increases.

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U. S. Fish and Wildlife Service, Microbiological Laboratory, Leetown, (P. O. Kearneysville), W. Va., and Salmon Nutrition Laboratory, Cook, Wash.

Fish were examined histologically from all four groups on Feb. 17, when the rate of mortality was climbing sharply in the first three groups. Moribund fish from Group A and nonmoribund specimens from Groups B, C, and D were selected. The fish were fixed in Bouin's solution for 24 hours and stored in 65% alcohol. The specimens were small, approximately 1 in. in length, and were embedded whole in paraffin, and longitudinal sections were made of the entire fish. Hematoxylin and eosin and Wolbach's Giemsa variant were used routinely.

Results

Only the moribund fish of Group A (Fig. 1) showed any histological evidence of pancreatic lesions, and all of these specimens were uniformly affected with acute pancreatic necrosis, as previously described.[1]

The fish of this group were affected also by necrosis of the voluntary muscles. The extent of the lesions varied from slight to extensive, but every moribund fish exhibited the lesion in some degree. Over half of the nonmoribund fish from Groups B and C of the infected populations also contained slight muscle necrosis. In these fish, very few muscle fibers were affected, however, and the disease was apparently in the initial stages. The severest lesions in Groups B and C were in a less advanced stage than those in the least affected fish from Group A. In the fish from Group D of the apparently noninfected population, two of six specimens were observed with a single muscle fiber in the first stages of hyaline degeneration.

The necrosis of the striated muscle fibers was segmented in type and resembled that described for Conn.-5 and Ohio-1 viral types.[6] The causative agent in this case was only moderately myopathic, and the lesion was limited to scattered fiber segments. In the severest cases, many more individual fibers were affected but the lesions were not generalized or widespread, as described for the Texas-1 strain.[6] Certain selected muscles exhibited a tendency for earlier involvement, particularly those of the lower jaw and the dorsal muscles of the neck. This was particularly true of the nonmoribund fish from the infected population. In the most advanced lesions, however, the majority of the striated muscles displayed at least a limited number of necrotic fibers.

The degeneration observed in the muscle fibers was a hyaline change similar to Zenker's degeneration. The condition apparently occurred with great rapidity, for only rarely were muscles seen in the early or intermediate stage of hyalinization. Almost invariably all internal detail of the fibers was obscured, and usually great distortion and crumbling of the hyaline masses had occurred. A few nuclear remnants were present, but such debris had often disappeared. There was no mineralization, and the sarcolemma was often intact.

In marked contrast to the sequence of regeneration recorded in mammals, practically no inflammatory or regenerative changes were observed. An occasional polymorphonuclear leukocyte was the extent of the inflammation. There was no "sarcolemmal tube" formation and few sarcoblastic slips. The picture was rather strikingly that of a normal muscle mass surrounding completely degenerated individual fibers, with only a barely perceptible reaction of the host tissue.

The livers of all three groups were moderately fatty, but there were no pathological changes. The hearts, central nervous systems, and adipose tissues were without distinguishing lesions.

Comment

Certain Coxsackie viral strains and the virus of mumps are the only known viral agents with the capacity to produce necrotizing pancreatitis.[6-8] The high incidence of pancreatic lesions in very young brook trout concurrent with necrosis of the striated musculature presents the interesting possibility that a relationship exists between the causative agent of this disease and the viruses of the Coxsackie group.

The lesions in fish bear a striking resemblance to those described in mice. The

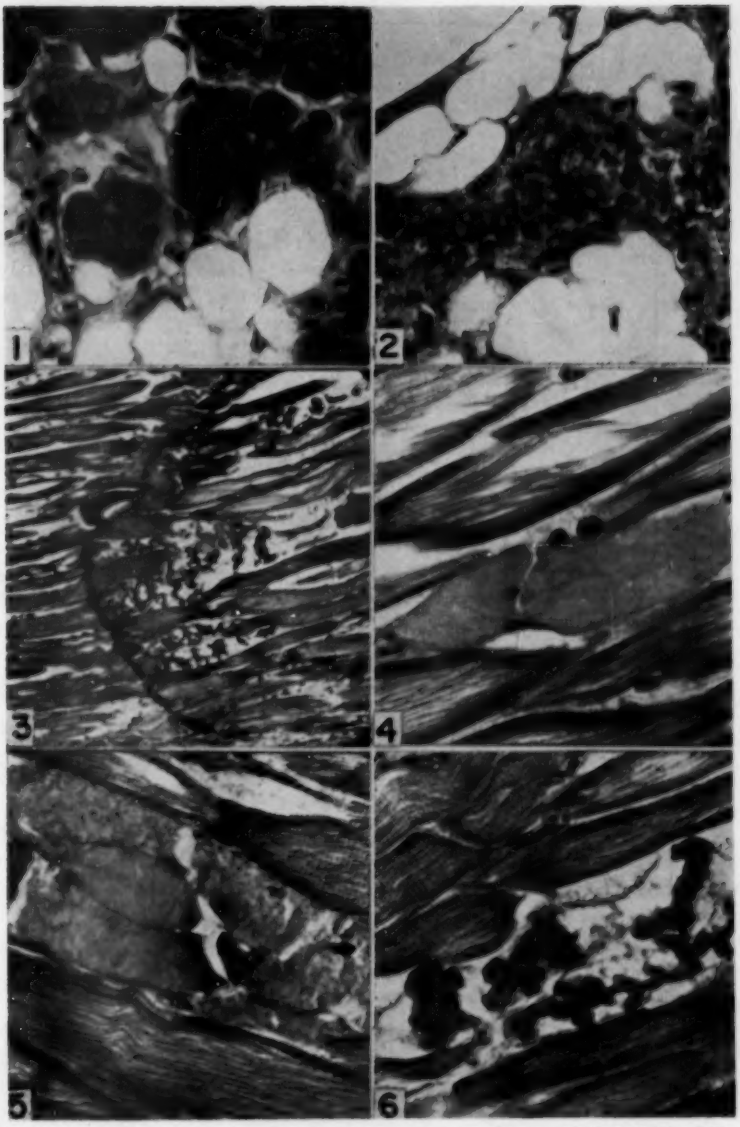

Fig. 1.—Normal acinar tissue of the brook trout pancreas from noninfected fish of Group A. Hematoxylin and eosin; × 475.

Fig. 2.—Acinar tissue in an advanced stage of necrosis from moribund fish of Group C. Hematoxylin and eosin; × 475.

Fig. 3.—Severe muscle necrosis in the caudal peduncle. Note the lack of inflammatory response. Hematoxylin and eosin; × 100.

Fig. 4.—A comparatively early stage of the hyaline degeneration. The surrounding fibers are normal. Hematoxylin and eosin; × 475.

Fig. 5.—The hyalinized bundles are beginning to crumple and fragment. Hematoxylin and eosin; × 475.

Fig. 6.—An advanced stage of crumpling and distortion. No inflammatory response has occurred. Hematoxylin and eosin; × 475.

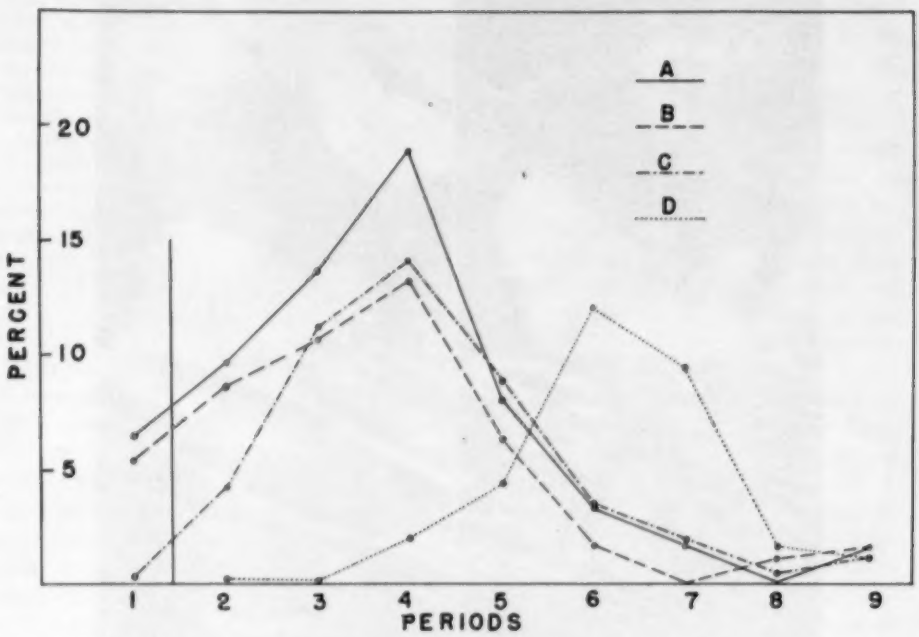

Fig. 7.—Percentage mortalities per five-day periods, starting on Feb. 10, 1955. The total losses, presented as percentages, during the time the records were kept (Feb. 10 to March 26, 1955) were as follows: *A*, 48; *B*, 40; *C*, 38; *D*, 28. Trout in Lots A and B were taken from hatchery troughs in which the disease was in full progress. In Lot C the disease just started, and those in Lot D were believed to be free from the disease on Feb. 10. The vertical line indicates the time (Feb. 17) at which specimens were removed for histopathological examination.

absence of inflammatory and regenerative changes in the muscle lesions is the most outstanding difference. This failure of repair may be due to the rapid death of moribund fish. Little is known of the processes of inflammation and repair in the salmonids, however, and the course of events may be much slower for this species. The occurrence of myopathy in nonmoribund fish from infected populations which had not yet developed pancreatic lesions suggests that the muscle lesions precede the pancreatitis. The very slight incidence of individual muscle fibers in early stages of hyalinization in fish from noninfected populations may indicate the very first stages of the disease or be evidence that the virus is endemic in these nonmoribund populations.

Our studies have not progressed to the point where we can successfully initiate the disease under laboratory conditions and observe the developing pathogenesis. It is possible that liver, heart, and brain injuries do develop which have so far been undetected.

Histopathological studies with fish have been in progress at the Salmon Nutrition Laboratory only since mid-1953. During this period, however, diseased fish have been received from all parts of the United States and several foreign countries for histological examination. Not infrequently severe noninflammatory myopathy of the type described in this paper has been observed. Since much of this material was fixed by untrained field personnel and was not accompanied by control specimens, the significance of the observations was open to question. There is, however, a growing body of evidence that fish are susceptible to or carriers for an infective agent or agents which produce lesions similar to those de-

scribed for the Coxsackie viruses. Since so little is known of the natural hosts of these organisms, the present observations should be of considerable interest.

Summary

A hyaline degeneration of the striated muscle fibers was observed in fingerling brook trout suffering from a disease described by us previously as infectious pancreatic necrosis. The histopathological changes in the pancreas and striated muscles, the infectious character of the disease, and the considerable susceptibility of a very young trout suggest the possibility that the disease may be caused by an agent having the characteristics of the Coxsackie group of viruses.

Microbiological Laboratory, Fish & Wildlife Service (P. O. Kearnysville).

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Rhabdomyosarcoma of Cerebellum

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The primary sarcomata of the brain are rare.[4,8,10] Nichols and Wagner [12] found an incidence of 0.6% in a group of 584 primary brain tumors. In 99 cases of primary intracranial tumors in adults, Earle [5] found 1 meningeal sarcoma and 3 hemangioblastomas. According to Ley and Rosendo [10] (1954), about 50 cases of sarcoma (including the meningeal sarcomas) have been published since the report of Bailey [2] (1929). Additional cases of brain sarcomata have been presented.[5,7,13] Excellent reviews of the older literature are available.[1,2,9] Recently, cases of rhabdomyosarcoma were reported in the bronchus [6] and ovary.[14]

The case to be reported of a primary rhabdomyosarcoma of the cerebellum in a woman is possibly the first case in the literature of such brain sarcoma.

Report of a Case

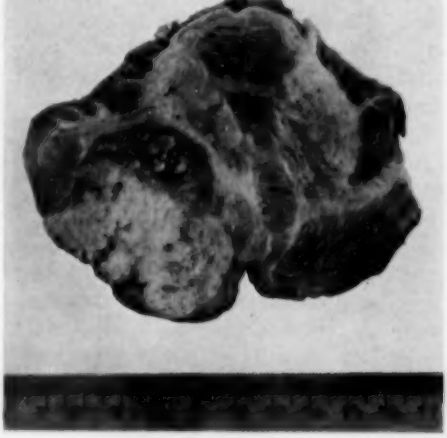
A woman aged 52 was admitted to the neurologic clinic on Aug. 7, 1952. She had complained of headache during the last five months; equilibrium disturbances appeared one and one-half months before the admission, so that the patient stood up with difficulty; marked akinesia appeared in the last 15 days, so that she had to lie in bed. The physical examination showed a well-nourished woman; pulse rate 135; arterial blood pressure 140/100 mm. Hg. Circulatory, respiratory, digestive, and urogenital systems without changes. On neurologic examination she was conscious but apathic, making difficult the investigation of voluntary motility. She moved the right arm with difficulty and could not sustain the limbs or stand up. She presented generalized deep hyperreflexia; the Babinski sign was absent. There was anisocoria; light and consensual reflexes were present, but slow. The cerebrospinal pressure was 200 mm. of water (suboccipital). The cerebrospinal fluid obtained there was normal. The electroencephalogram showed very irregular electric brain activity on both sides. Roentgenograms of the skull and angiography through the right carotid artery were normal. During the angiographic test through the left carotid artery the patient died, owing to respiratory arrest.

Necropsy (carried out three hours after death)

The body was that of a well-nourished woman 58 kg. in weight and 165 cm. in length. The cerebral dura mater was distended. The brain was voluminous (1200 gm.), with conspicuous signs of compression due to increased intracranial pressure (herniated cerebellar tonsils, flattening of the anterior surface of the medulla oblongata, etc.).

Within the left enlarged cerebellar hemisphere there was a hard tumor, slightly salient at the surface of the posterior cerebellar border. This tumor was covered by the leptomeninges and was not adherent to the dura mater. A horizontal section through the posterior cerebellar border showed a round, well-circumscribed, white tumor, 5 cm. in diameter, which replaced there the gray and white cerebellar substance and compressed the adjacent cerebellar parts. The tumor showed a wide necrotic area in its anterior portion (Fig. 1). Evidence of true encapsulation failed. Leptomeninges presented only hyperemia. The other organs showed no

Fig. 1.—Tumor within the left cerebellar hemisphere.



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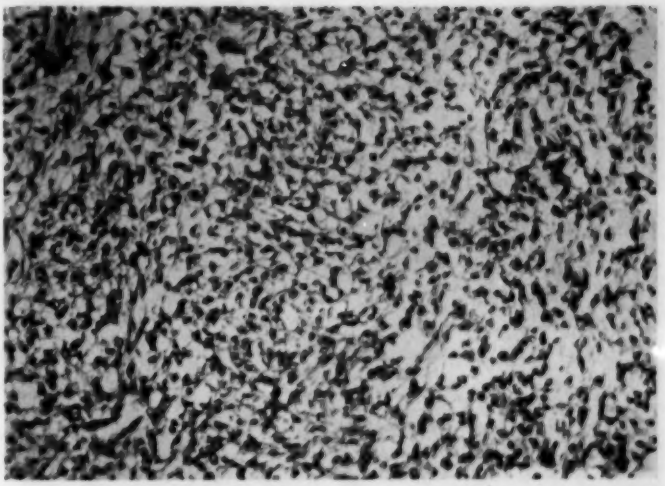

Fig. 2. — Undifferentiated area of the tumor. Cells of the mesenchymal type form a loose syncytium. Hematoxylin-eosin stain; reduced to 8/9 of mag. × 300.

changes, except wide fibrous adhesions of the right lung to the chest wall, diffuse acute vesicular emphysema of the lungs, slight atherosclerosis of the aorta and coronary arteries, atrophy of the ovaries, and three small typical uterine leiomyomas, the largest of which measured 1 cm. in diameter. Two of these myomas were subserous and one intramural. Lymph nodes without changes.

Microscopic Examination

The histology varied markedly in sections prepared from different parts of the tumor and in the same section, so that three patterns could be observed. In the first the cells were of mesenchymal type, disorderly arranged, uniform in appearance, stellate in shape, and forming a loose syncytium through fusion of their processes (Fig. 2). The nuclei, changing little in size, were vesicular and generally rounded or elongated, with frequent conspicuous nucleoli; the cytoplasm was not abundant and was constituted mainly of the cellular processes; hyperchromatic nuclei and mitoses were rare. This tissue presented very infrequent reticular fibers.

In the second pattern the mesenchymal cells were elongated and densely packed, with a tendency to form bundles; there the reticular fibers were more frequent. In a few bundles a conspicuous fibrosarcomatous

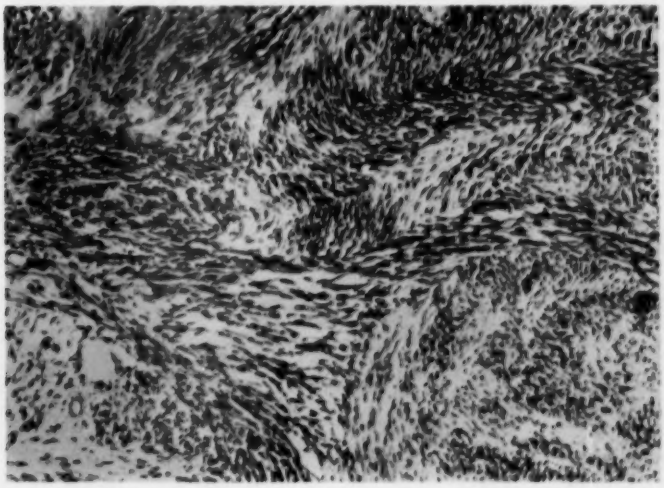

Fig. 3.—More differentiated area of the tumor, in which the cells form bundles with smooth and striated muscle cells. Hematoxylin and eosin stain; reduced to 8/9 of mag. × 100.

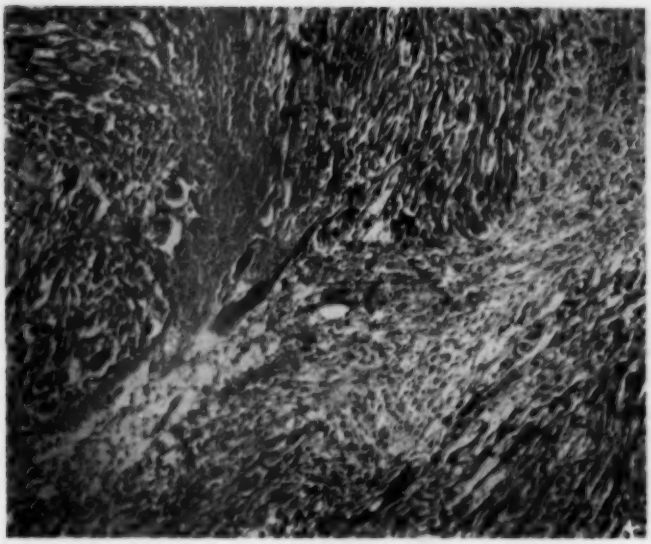

Fig. 4.—Rhabdomyosarcomatous pattern with necrotic area (at left), myomatous giant cells and fibrosarcomatous area (lighter tissue at right). Hematoxylin and eosin stain; × 100.

feature was seen, due to differentiation of collagenous fibers between the neoplastic cells (Fig. 4). These areas were less cellular and rich in fibers, staining lighter with hematoxylin and eosin. The number of collagenous fibers, however, changed, and all the transitional features between the undifferentiated mesenchymal tissue and the fibrosarcomatous one were seen.

In the third pattern the cells were elongated and formed conspicuous bundles in several directions. These bundles presented fusiform cells with scarce, nonstriated cytoplasm and striated muscle cells, as well as transitional forms between these cell types. There were bundles in which the smooth cells predominated and others in which the striated muscle cells predominated (Fig. 3). The striated cells changed in size and form (rounded, tadpole-shaped, fusiform); frequently, however, they were long and well differentiated, in fascicular arrangement, with central or peripheral nucleus, strongly eosinophilic cytoplasm, and distinct cross striations even in the hematoxylin and eosin stain (Fig. 5). Frequent rounded or

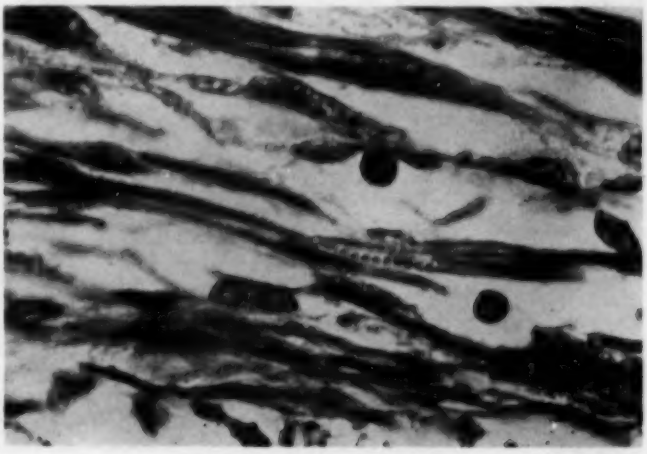

Fig. 5.—Rhabdomyosarcoma. Striated muscle cell. Mallory's phosphotungstic acid-hematoxylin; × 950.

elongated multinucleated striated muscle cells were noted. The cytoplasmic content of the transitional cells changed, as well as its staining with eosin, there being found cells with faint, scarce, or more abundant eosinophilic cytoplasm, provided or not with longitudinal striations. In these areas with muscle differentiation the malignant features were more prominent. There a frequent nuclear hyperchromatism, mitoses, many multinucleated giant cells, with or without striations, and necrotic areas were seen (Fig. 4). Also, undifferentiated polymorphic, rounded, ovoid, and fusiform cells, varying greatly in size, were present in these areas. On the other hand, rare muscle cells with and without striations were detected in the undifferentiated areas. The reticular network was conspicuous in the muscle-cell bundles. The neoplastic tissue was provided with many blood vessels. The adjacent leptomeninges and nerve tissue presented infiltration with the neoplastic cells.

Comment

The histological picture in this case was sufficiently typical of rhabdomyosarcoma to make the diagnosis certain. The differentiation of striated muscle fibers from undifferentiated mesenchymal tissue was conspicuous. All the transitional cell types, from the undifferentiated mesenchymal cells to nonstriated and striated muscle cells, were seen. The changes in the sense of differentiation of the latter were detected through the cellular enlargement, the degree of staining by eosin, and the appearance of striations in the cytoplasm of the cells (Figs. 3, 5). Intermingled with the myosarcomatous and the undifferentiated tissues were a few fibrosarcomatous areas. Borst[3] pointed to a sarcomatous stroma in the rhabdomyosarcomas and emphasized that these frequently are mixed tumors. However, we regard the fibrosarcomatous areas as tissue differentiated from the undifferentiated mesenchymal elements, because conspicuous transitional features were seen.

The histogenesis of the neoplasm is a matter for speculation. It arose primarily within the cerebellum, no other primary tumor being found in the body. The matrix tissue may be misplaced embryonic rests or undifferentiated mesenchymal cells arranged along the blood vessels (Maximow and Bloom[11]) of the pia mater or of the cerebellum. The origin from mesenchymal cells is argued by Willis[15] as a most probable source of rhabdomyosarcomatous tumors. Willis emphasizes the striking fact that rhabdomyosarcomatous tumors are much commoner in sites where normally no striated muscle is present. The adult age of our patient, the undifferentiated mesenchymal areas, and the fibrosarcomatous ones, as well as the accepted origin of other brain sarcomas from undifferentiated mesenchymal perivascular cells,[7] suggest that this rhabdomyosarcoma arose from the last-mentioned cells and not from embryonic rests. A teratomatous origin is to be discarded, because tissues other than the aforementioned were not found in the sections.

The first symptom in our patient was headache, which appeared five months before death. Therefore the course of the tumor was rapid. Metastatic growth within or outside the intracranial cavity was not found. The same observation is made with other intracranial sarcomatous tumors.[9]

I did not find in the available literature any case of a primary rhabdomyosarcoma in the brain. Moreover, the histological classifications of primary brain sarcomas did not include the rhabdomyomatous type.[1,12] Henschen,[8] however, presented a secondary malignant rhabdomyoma of the cerebellum originating in the epipharynx.

Summary

A case of primary rhabdomyosarcoma of the cerebellum in a 52-year-old woman is presented. Possibly this is the first rhabdomyomatous sarcoma to be presented in the brain. The histogenesis of the tumor is discussed, and an origin from undifferentiated mesenchymal perivascular cells is preferred.

The clinical data were supplied by the neurological clinic of this Faculty of Medicine, by courtesy of Prof. Dr. A. Tolosa.

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Accumulation of Mast Cells in Endosteum of Bones of Calcium-Deficient Rats

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New and interesting material for study of the mast cell was encountered in the course of experiments dealing with rats fed on a diet low in calcium. The experiments were designed originally to observe growth and repair of bone, including the process of calcification of cartilage and bone matrix. The procedure consisted of correlating the serum calcium and phosphorus with the appearance of undecalcified sections of the tibia of animals at intervals of 21 to 130 days of age. Mast cells were seen in the bones by chance as a result of staining with hematoxylin-eosin-azure II.[6] This method, now used routinely in our studies, has been most helpful for distinguishing the great variety of connective tissue cells present in growing bone marrow and bone tissue. The azure II component stains the large granules in the cytoplasm of the mast cell in a form that is fast and insoluble in water.[5] Hematoxylin and eosin, the stain generally used for histological examination of bone, does not disclose the presence of mast cells. Observations of mast cells in the bones will be presented in this report chiefly by means of numerous photomicrographs and with only the minimum of descriptive material. The experiments will be presented without any attempt to deal with the details of the cytological or histochemical aspects of the subject. The work dates back to 1937 and is reported at this time because of the great interest in the subject of mast cells in the current literature in all fields of biology and medicine.

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Department of Surgery, Division of Orthopedics, and The Atomic Energy Project, University of California, at Los Angeles, and the Department of Physiology, University of Chicago.

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Materials and Methods

Two hundred white laboratory rats, Wistar Institute strains, were weaned at 21 days of age to a calcium-deficient diet described by Shohl as Diet E. The ingredients of the basic diet were those of the Hess and Sherman modification of Steenbock and Black's rachitogenic Diet No. 2965; i. e., 79% corn meal, 20% gluten, and 1% NaCl.[21] The calcium content of this mixture, according to Shohl, was 0.05%-0.06%, and the phosphorus content, as phosphate, was 0.12%. To prepare Diet E no calcium was added, but the phosphorus content was brought to 1.00% by addition of the appropriate amount of KH_2PO_4 (3.85 gm. per 100 gm. of basic mixture).

The content of vitamin D of this diet, as well as that of calcium, was most inadequate for sustaining growth and calcification of the skeleton. The effect of the diet on the skeleton was unique, in that it produced (1) bone atrophy (osteoporosis), (2) a form of rickets, contributed to by the deficiency in vitamin D, as well as that in calcium, and (3) osteitis fibrosa, presumably resulting from hyperactivity of the parathyroid glands secondary to the deficiency in vitamin D and in calcium. Rickets was difficult to detect by x-ray because the thickness of the epiphyseal plate was increased slightly, if at all.

The characteristics and effects of Diet E on the rat have been described by Shohl. The Ca/P ratio was 1:16; Shohl's control diet contained 1.0% calcium and 0.5% phosphorus, with a Ca/P ratio of 2:1. Diet E was markedly acid (285 cc. of 0.1 N titratable acid per 100 gm. of diet). Shohl found a bone ash content, after 21 days on the diet, of 32%, with serum calcium 3.9 mg. per 100 cc. and serum phosphorus 7.6 mg. per 100 cc., as compared with values of 46% for bone ash, 10.0 mg. per 100 cc. for serum calcium, and 7.6

TABLE 1.—*Treatment and Results in Fifty-Five Young Growing Control and Experimental Animals Without Fractures, 21 to 59 Days of Age**

Group	No. Rats	Diet	Days on Diet	Other Treatment	Mast Cell Counts in Bone Field per High-Power
I	10	Normal (Bill's)	1 to 31	None	0-1
II	8	Calcium deficient	2 to 16	None	0-3
III	19	Calcium deficient	17 to 21	None	0-5
IV	7	Calcium deficient	26 to 37	None	1-6
V	2	Calcium deficient	31 to 32	None	25-50
VI	9	Calcium deficient	27 to 38	Parathyroid injection†	25-75

* The mast-cell count per high-power field was proportional to the period of time on the deficient diet. Parathyroid injection U.S.P. probably had no specific effect, but a large number of mast cells were retained in the fibrous bone marrow or area of osteitis fibrosa produced by the parathyroid hormone.
† 100 I. U. per day for five days, four rats; 200 I.U. per day for five days, five rats.

mg. per 100 cc. for serum phosphorus in rats on the control diet.

Our rats were reared on the experimental diet for from 2 to 105 days. In 145 rats a closed fracture of the tibia was produced manually after they had been fed on the calcium-deficient diet for at least 21 days (Table 2). The animals were killed at three-day intervals of healing. The upper end of the tibia and the lower end of the femur were examined for comparison in 55 rats without fractures but similarly fed the experimental diet and prepared for blood and histological studies (Table 1). Twelve rats without fractures and 20 rats with fractures, litter mates of the animals reared on the calcium-deficient diet, were fed a standard diet (Fox Chow) to provide the blood and bone of normal controls (Tables 1 and 2).

The bones were sectioned undecalcified with the soft parts attached, thus providing muscle, fascia, and synovial membrane for mast-cell counts. Mesentery was excised and stained separately with toluidine blue or neutral red as whole mounts. All of the mast-cell counts were made on high-power fields (× 450).

Serum calcium was determined on pooled sera of each litter by Clark and Collip's modification of the method of Kramer and Tisdall; serum inorganic phosphorus was determined by the method of Fiske and Subbarow, as used in our previous studies on rachitic rats.[18,19]

Results

Normal white rats reared in our laboratory reached a plateau in their rate of growth at 12 to 14 weeks of age. Litter mates weaned to Shohl's Diet E at 3 weeks of age showed almost complete arrest of growth at 7 weeks of age. The animals consumed large quantities of food but gained little, if any, weight. After only one week on this experimental diet there was already significant retardation of growth. After three to four weeks growth of the

TABLE 2.—*Treatment and Results in 155 Young Control and Experimental Animals, 21 to 127 Days of Age, with Fractures of Right Tibia from One to 106 Days of Healing**

Group	No. of Rats	Diet	Days on Diet	Other Treatment‡	Mast-Cell Counts per High-Power Field
I	20	Normal	21 to 63	None	0-2
II	10	Calcium-deficiency rickets	21 to 28	None	25-35
III	10	Calcium-deficiency rickets	21 to 35	None	25-50
IV	5	Calcium-deficiency rickets	21 to 46	None	25-100
V	15	Calcium-deficiency rickets	21 to 49	None	25-150
VI	10	Calcium-deficiency rickets	21 to 49	None	25-150
VII	5	Calcium-deficiency rickets	28 to 106	None	50-200
VIII	10	Calcium-deficiency rickets	21 to 49	Sodium phosphate	25-150
IX	10	Calcium-deficiency rickets	21 to 49	Calcium chloride	25-150
X	15	Calcium-deficiency rickets	21 to 106	Vitamin D	25-150
XI	15	Calcium-deficiency rickets	21 to 40	Parathyroid injection U.S.P.	25-100
XII	10	Phosphorus-deficiency rickets†	21 to 49	None	0-2
XIII	10	Beryllium rickets‡	21 to 49	None	0-2
XIV	10	Strontium rickets§	21 to 49	None	0-2

* Mast cells appeared in cancellous bone everywhere in the skeleton. Mast-cell counts show that the number of cells per high-power field was proportional to the period of time on the calcium-deficient diet. Litter mates fed control diets and various rachitogenetic diets not deficient in calcium showed no increase in the number of mast cells in cancellous bone. Vitamin D produced absorption of osteoid tissue and the mast cells. Other forms of treatment had no effect on mast cells.
† Steenbock-Black Diet #2965.
‡ Normal diet plus 2% beryllium carbonate.
§ Normal diet plus 4% strontium carbonate.

tibia in both length and diameter had been suppressed and actually brought to a standstill. Many of the animals showed no increase in weight even after four to six weeks. A typical rat weighing 25 gm. at the age of 21 days weighed 25 gm. at 63 days of age. The nose-to-tail length, as well as the height of the rat, was approximately one-half of normal. The stunting was readily apparent, of course, in all parts of the body, not just the bones, but the effects were more easily visible in the skeleton than elsewhere. Shohl and many other observers have described certain aspects of the mineral metabolism and rickets developed in rats on diets with various degrees of calcium deficiency. The bone tissue and the bone marrow will be described here briefly in relation to the appearance of mast cells.

Three weeks after a rat was on the diet low in calcium, mast cells in numbers as high as 5 to 10 per high-power field were found on the outer surface of the layer of osteoblasts. At first, they were elongated, rectangular, or fusiform in shape, rather than ovoid, as in the mesentery. After four weeks the osteoblastic layer reverted to the resting form and mast cells were found between the connective tissue cells and around blood vessels in numbers as many as 25 per high-power field. After 6 to 15 weeks, the numbers increased to from 50 to 200 per high-power field, and mast cells were seen everywhere throughout the endosteum, and occasionally even in direct contact with osteoid tissue. At this stage they were ameboid and of all shapes—round, triangular, and oval—with cytoplasm completely filled with large deep-blue and metachromatic-staining granules.

Mast Cells in Soft Tissues.—The new location and the increase in numbers of mast cells was not preceded by any change in the normal population of mast cells in the primary marrow cavity of the same bone. Increase in the mast cells in the marrow of cancellous bone appeared to follow rather than to precede the increase in mast cells in endosteum. However, the mesentery, myomysium, synovial membrane, and other tissues did not show greater numbers of mast cells than were seen in normal rats. There were some large clusters of mast cells in connective tissue around small blood vessels in the typical perivascular arrangement that is commonly seen in the fascial planes and myomysium in rats. It was difficult, by cell counts on ordinary histological sections, to determine whether there was any increase in the number of cells in the clusters in muscle and fascial planes because of the irregularity and haphazard distribution of mast cells in soft tissues.

Blood.—In animals reared on Shohl's Diet E in our laboratory the total serum calcium was 1.26 to 1.63 mM. per liter and the inorganic phosphorus was 2.04 to 3.21 mM. per liter of the pooled sera of litter mates. In litter mate rats reared on control diets the serum calcium was 2.50 mM., and the serum inorganic phosphorus was 2.3 mM. per liter. The concentration of calcium in the serum in the experimental animals was thus approximately half that of the serum of normal animals. The concentration of phosphorus was approximately the same as, and occasionally even higher than, that in the serum in normal rats. The concentration of these elements in the serum was thus, as observed by Shohl, roughly proportional to the amount in the diet.

Bone.—A peculiar combination of rickets and bone atrophy (osteoporosis) developed in rats fed the experimental diet that was markedly deficient in calcium but high in phosphorus. The calcification mechanism lagged behind the growth of the epiphyseal cartilage, and the new bone tissue was always only partially impregnated with mineral. Calcification did not cease entirely, as it does in animals on rachitogenic diets high in calcium and low in phosphorus.

Two weeks after young rats were weaned to Shohl's Diet E, the epiphyseal plate was generally thicker than normal and the bone trabeculae were only partially calcified and

coated with osteoid. At three to four weeks the character of the osteogenetic and myeloid connective tissue showed a striking change. The osteoblasts, normally large polygonal deeply basophilic cells, reverted to a spindle-shaped resting form. Osteoclasts became more numerous. The bone trabeculae in the metaphyseal spongiosa were half the normal number and length. The marrow reticulum between the bone trabeculae consisted of fibrous connective tissue cells with large vascular spaces and much less hemopoietic tissue than normal.

After four to eight weeks on the diet, a highly complex pathological picture appeared throughout the skeleton, particularly in the metaphyseal regions. Wolbach noted in grading the rickets produced by various diets by Shohl that there were special effects and unusual thinning of the epiphyseal plates in the rats reared on Diet E even for a relatively short time.[11] He did not describe these bones further, other than to mention that they were different from those of rats on any of the other rachitic diets. Briefly stated, our observation is that Diet E produced a combination of moderate rickets, bone atrophy (osteoporosis), and osteitis fibrosa. The rickets, a manifestation of the inadequate amount of vitamin D in the diet, was evident in the irregularity or lack of calcification of the epiphyseal plates and the osteoid tissue in the metaphyses. The bone atrophy (osteoporosis) was seen in the thinning and reduced number of bone trabeculae per unit area or volume of bone spongiosa, and especially in the metaphyseal region bordering on the marrow cavity. The osteitis fibrosa was also seen throughout the secondary spongiosa in the form of a relatively large amount of fibrous tissue, with loss of the cords of normal bone marrow between the new bone trabeculae. There were spindle-shaped fibrous connective tissue cells in the endosteum in areas normally occupied by osteoblasts in growing rats. In addition, there were increased numbers of osteoclasts. These changes in the bone marrow seem not to have been observed in rats on low-calcium diets. Hypertrophy of the parathyroid glands, however, has been described in animals on low-calcium diets. It is likely that the osteitis fibrosa aspect of the pathological picture produced by Shohl's Diet E results from stimulation of the parathyroid glands by the low level of calcium in the tissue fluids. Further investigation of hypersecretion of parathyroid hormone is necessary in animals fed diets as low in calcium as Shohl's Diet E. The finding pertinent to this report is that mast cells appeared in the fibrous tissue in the bone marrow that is characteristic of osteitis fibrosa. After four weeks on the diet mast cells appeared in the perivascular elements outside the cords of bone marrow. After six to eight weeks on the diet (in rats 9 to 11 weeks of age) mast cells were found inside, as well as on the surface of, the endosteum. The change was most extreme

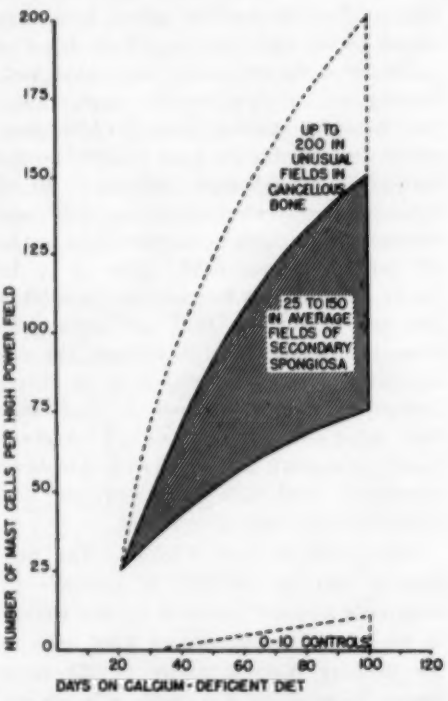


Fig. 1.—Graph showing the relationship between the mast-cell counts and the period of time the animal was reared on a calcium-deficient diet.

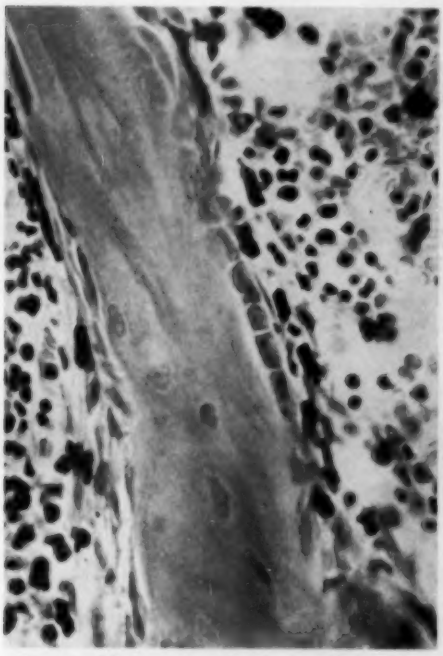


Fig. 2.—Photomicrograph (reduced to 62% of mag. × 900) showing rectangular or spindle-shaped mast cells formed in a row in the layer of connective tissue cells on the outer surface of the osteoblasts. The cuboidal cells on the right side of the bone trabecula are osteoblasts. A spindle-shaped "resting" form of osteoblasts is seen on the left side. Seven-week-old rat fed on Diet E for four weeks. Hematoxylin-eosin-azure II stain.

in the attenuated cancellous bone throughout the secondary spongiosa.

Mast Cells Near Bone Tissue.—The origin of the mast cell in the bones appeared to be the same as it is generally believed to be in other locations, that is, from undifferentiated young connective tissue cells. If mast cells are capable of ameboid locomotion, as some cytologists now believe, it is possible that they are formed in the marrow and migrate into the endosteum. In normal young rats, an occasional mast cell was seen near the endosteum. Mast cells were not found near bone tissue in rats with phosphorus-deficiency rickets, strontium rickets, or beryllium rickets (Table 2; Figs. 1 to 8).

Effects of Vitamin D, Parathyroid Hormone, and Parenteral Injections of Calcium Chloride or Sodium Phosphate

Vitamin D.—The most effective method of treatment of calcium-deficiency rickets was oral administration of vitamin D. One litter was treated with calciferol U. S. P. (irradiated ergosterol), approximately 900,000 I. U. of vitamin D activity per cubic centimeter, for three to seven days. The result of this treatment was that the rachitic metaphysis was promptly revascularized and absorbed; the epiphyseal cartilage developed a new zone of provisional calcification and a new primary spongiosa. The denser bone of the secondary spongiosa was little changed during the relatively brief period of one week of treatment, whereas the upper metaphysis showed striking alterations. The sequences of changes in the mast cells in the reacting area of the bone were as follows: (1) The osteoid and bone trabeculae were resorbed, together with their adnexa of fibrous tissue and mast cells; (2) the density, number, and size of the granules in the cytoplasm of the mast cells were diminished, and the clear light-blue-staining nucleus of the cell was revealed; (3) the bone marrow became hemopoietic and the mast cells disappeared into it. At the edges of this area of healing there were dense clusters of mast cells, 50 per high-power field; some of these had reverted to a spindle-shaped connective tissue form. At the same time there was thinning and loss of the cytoplasmic granules.

Parathyroid Injection U. S. P.—Two litters of rats were treated with intramuscular parathyroid injection U. S. P. 50 to 1000 units per day for a period of seven days. This form of treatment could only initiate calcification in the form of a band across the hypertrophic cartilage cells of the rachitic epiphyseal plate; it could not restore the bones to a normal condition. Instead, it superimposed another pathological process. Across the upper metaphysis a disc of osteitis fibrosa was formed in place of the primary spongiosa. The interior of this

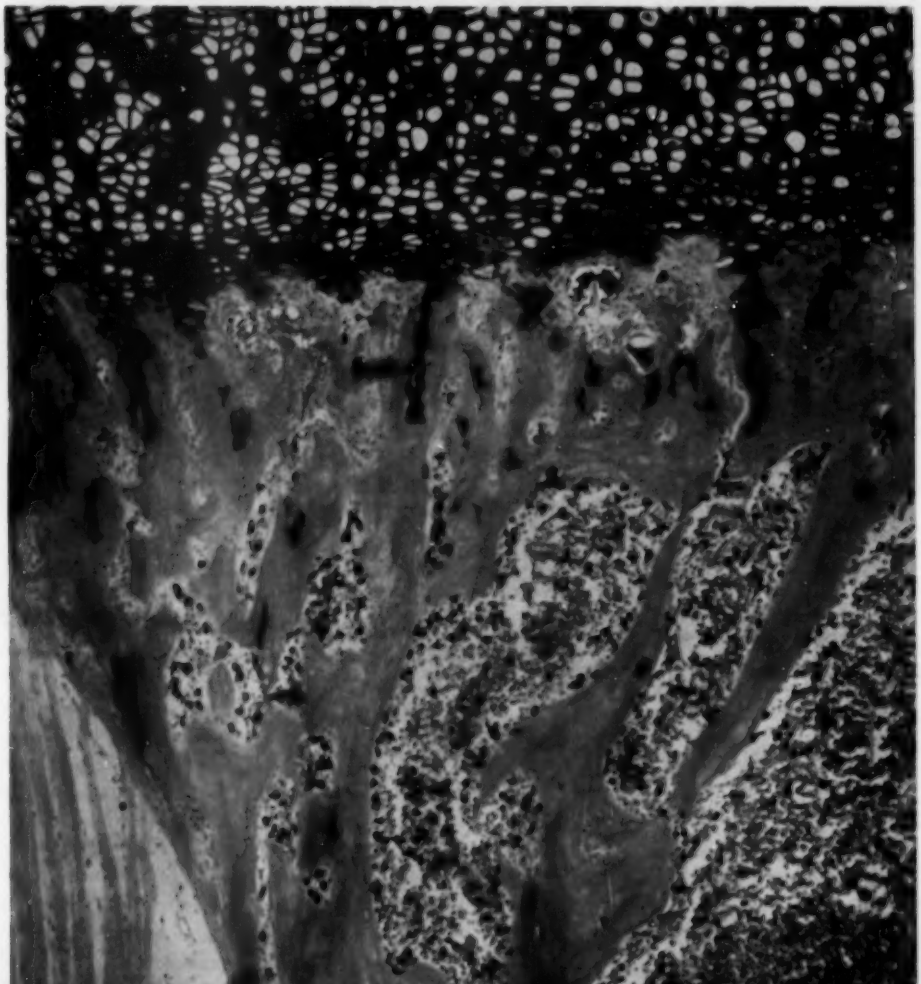


Fig. 3.—Photomicrograph (reduced to 68% of mag. ×200) showing the upper end of the tibia of a rat, 8 weeks of age, reared on a calcium-deficient diet for four and a half weeks. The epiphyseal cartilage appears across the top of the picture; muscle and periosteum, on the left; bone marrow, on the right. Note the deeply stained mast cells within and upon the endosteal lining of bone trabeculae of secondary spongiosa and of the inner lamella of the new Haversian canals. Hematoxylin-eosin-azure II stain.

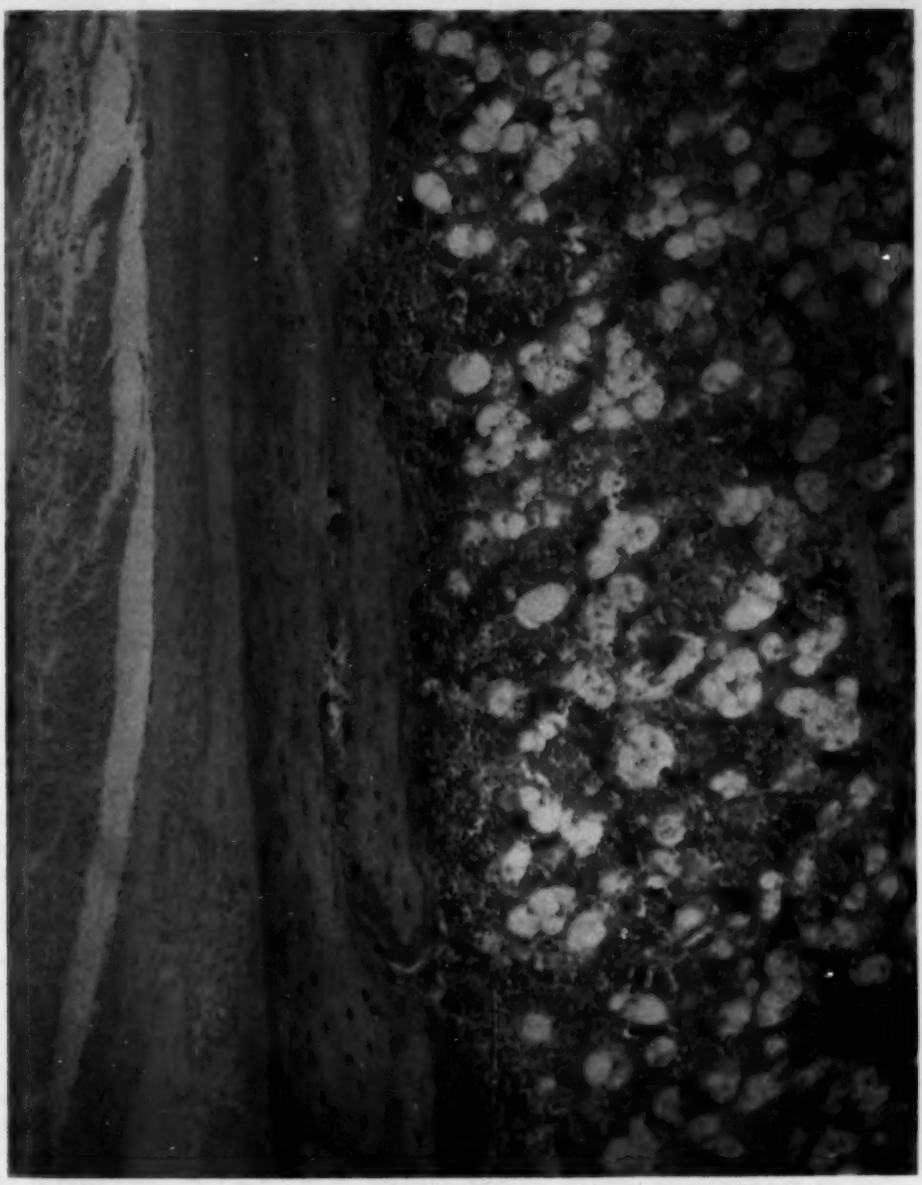


Fig. 4.—Photomicrograph (reduced to 68% of mag. ×750) showing mast cells among endosteal cells lining the shaft of the tibia. A large Haversian canal in the center of the compact bone also contains mast cells. Subperiosteal new-bone formation from an eight-day healing fracture is shown at the lower left-hand side of the picture. In that location, where there is vigorous new-bone formation, there are no mast cells. Hematoxylin-eosin-azure II stain.

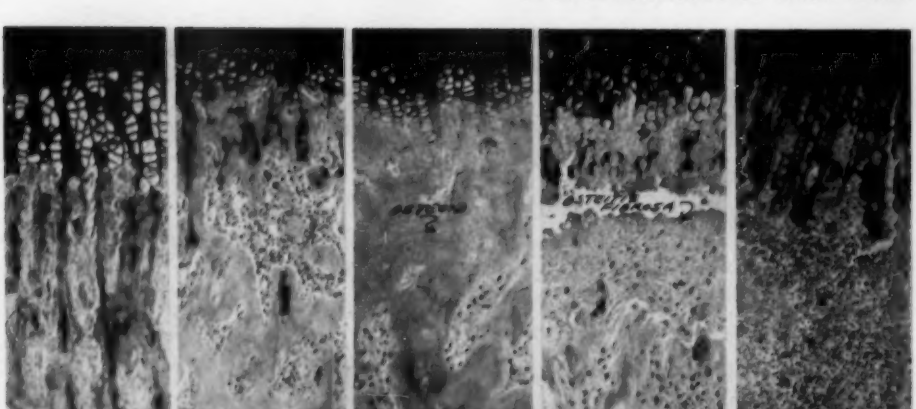


Fig. 5.—Photomicrographs (reduced to 52% of mag. ×75) showing the appearance of the metaphysis of animals fed on the experimental diet: *A*, 31-day-old rat after 10 days on Diet E, showing many cuboidal osteoblasts and thin osteoid borders but no mast cells near bone tissue; *B*, 52-day-old rat after 31 days on Diet E, showing very few cuboidal osteoblasts, much more osteoid, and mast cells in bone marrow and endosteum; *C*, 74-day-old rat after 53 days on Diet E, showing no osteoblasts, more osteoid, and many mast cells; *D*, 64-day-old rat after 43 days on Diet E and administration of 5000 I. U. of parathyroid injection U. S. P. in five days, showing new calcification in epiphyseal cartilage matrix, osteitis fibrosa across the upper metaphysis, and very large numbers of mast cells; *E*, 57-day-old rat after 36 days on diet treated with 500,000 I. U. vitamin D in five days, showing new calcification of epiphyseal cartilage, absorption of rachitic metaphysis, formation of new bone marrow, and dispersal and reduction in size and number of mast cells.

area retained a large number of mast cells. Mast cells were also present in the cancellous bone of the secondary spongiosa in numbers as great as in the untreated rats (Fig. 5).

Parenteral Injections of Calcium Chloride or Sodium Phosphate.—One litter of rats was treated with intraperitoneal injections of a 100 mM. solution of calcium chloride, 1.0 ml. per 100 gm. of body weight. One litter of rats was treated with intraperitoneal injections of a 100 mM. balanced solution of sodium phosphate ($Na_2HPO_4 \cdot NaHPO_4$), 2.5 ml. per 100 gm. of body weight.

In animals with a long-standing calcium-deficient diet, neither of these forms of treatment produced any appreciable reversal of the rachitic process, and they had no effect on the number or location of mast cells. Sodium phosphate injections, in doses capable of curing phosphorus-deficiency rickets, either accentuated or had no effect on the pathological process in calcium-deficiency rickets.

Fractures

Healing fractures in calcium-deficient rats were examined to determine whether mast cells developed from any of the elements of connective tissue that are normally used for bone formation. It was concluded that mast cells, bone marrow, and bone tissue had a common origin in undifferentiated perivascular young connective tissue cells in fracture callus, the same as in the metaphysis. In from 1 to 21 days of healing, when the callus was rapidly growing in size, there were no mast cells near bone-forming connective tissue inside the fracture site. Mast cells were not seen in the fibrocartilaginous callus. They were seen in the endosteum and bone marrow of the mature periosteal bone deposits in numbers and intervals as follows: 0 to 2 per high-power field at 14 days; 0 to 5 at 19 days; 10 to 20 at 24 days; 25 to 30 at 36 days; 50 to 200 at 42 days. After the callus reached its maximum size, the bony portion showed cords of fully developed hemopoietic marrow between the

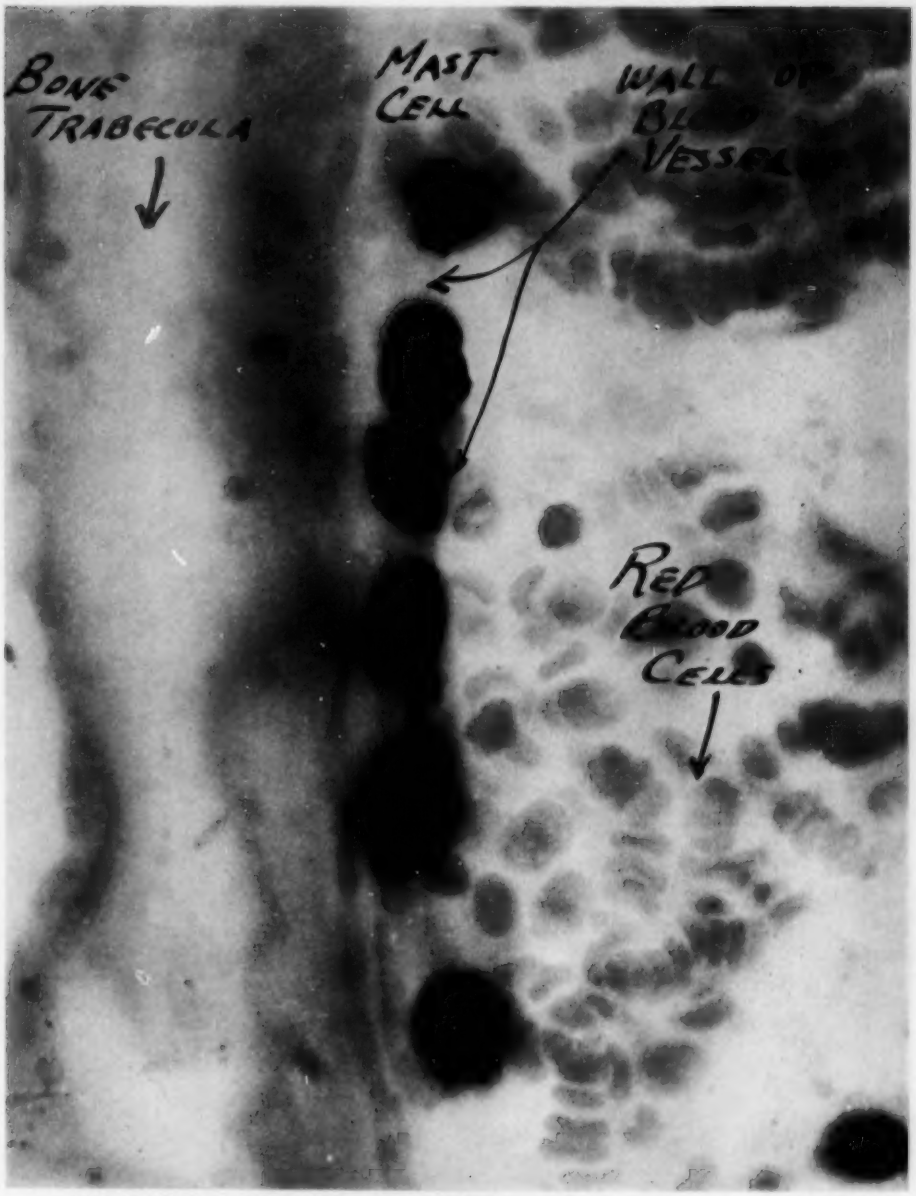


Fig. 6.—Photomicrograph (reduced to 71% of mag. ×2000; oil-immersion field) showing mast cell as it appears in endosteum in a calcium-deficient rat 8 weeks of age, after 35 days on the experimental diet.

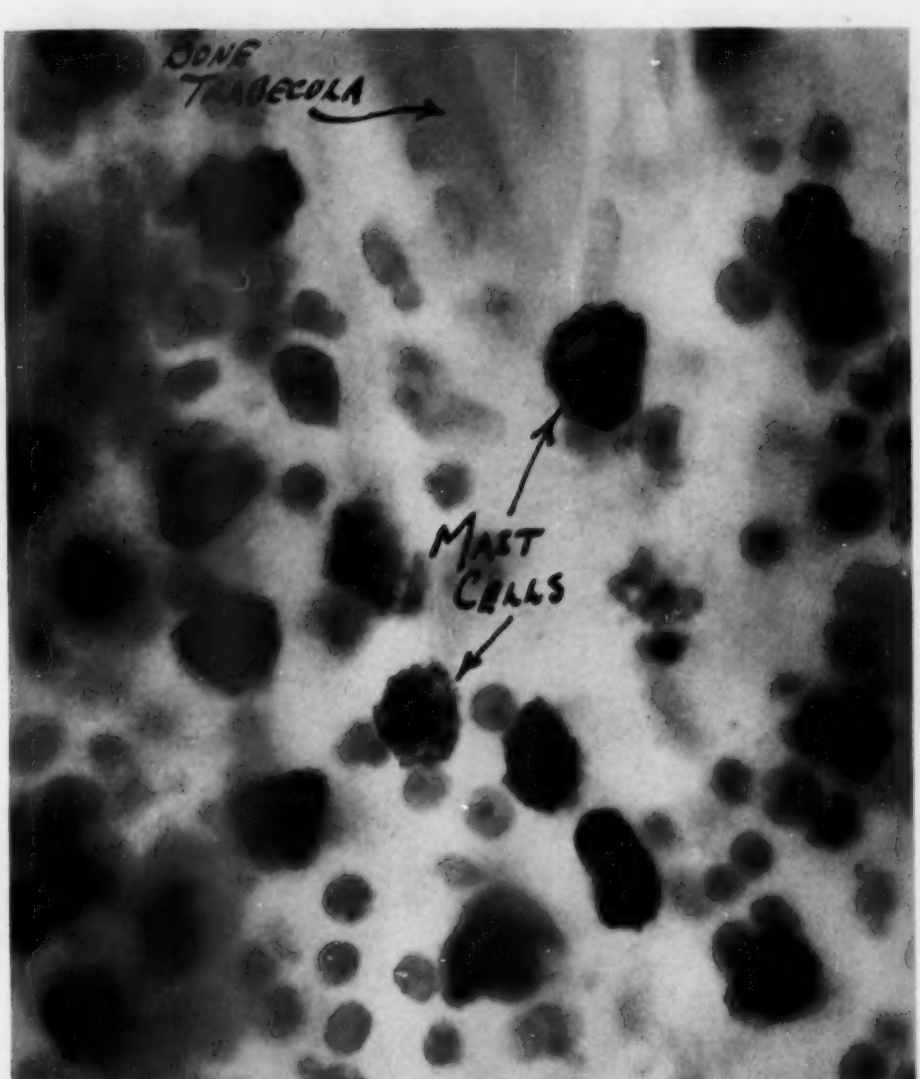


Fig. 7.—Photomicrograph (reduced to 68% of mag. × 2000; oil-immersion field) showing a mast cell on the edge of an area of absorption of osteoid following treatment of a 57-day-old calcium-deficient rat with 500,000 I. U. of vitamin D. The granules are fewer, smaller, and spread apart, and the nucleus of the cell is revealed more clearly.

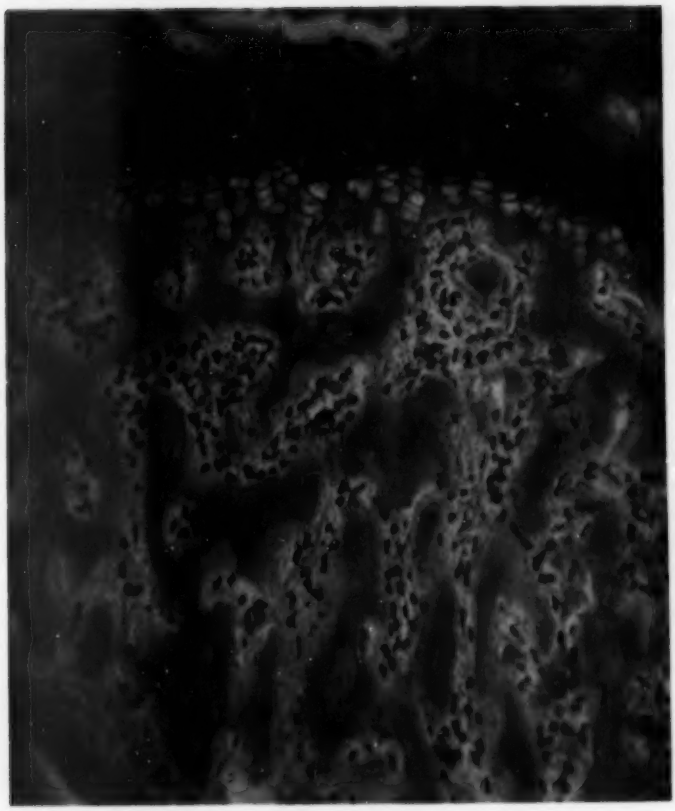


Fig. 8.—Kodachrome (enlarged ⅓ from mag. × 150) showing extraordinary increase in mast cells throughout the metaphysis in bone marrow and endosteum in 70-day-old rat after 49 days on the experimental diet.

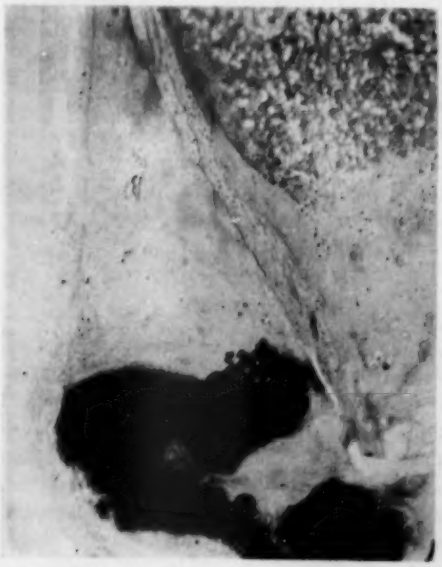
bone trabeculae. At the same time, the character of the endosteum changed from polygonal-shaped osteoblasts to fusiform connective tissue cells, and it was then that mast cells appeared in the callus in very large numbers, 50 to 200 per high-power field, the same as in the metaphysis of the same bone.

In one experiment, including 10 animals, the metaphysis of the fractured tibia was compared with that of the opposite, unfractured tibia. The mast cells were quite as numerous in the sound limb as in the fractured one; this excluded any possibility that inflammation produced by the fracture could cause the accumulation of mast cells in a bone (Figs. 4 and 9).

Comment

The experiments described in this report offer additional material with which investigators may search for the solution to the "riddle of the mast cell."[9] The literature has recently been reviewed in detail by Riley. On the basis of his own and his collaborators' work, and of papers he analyzed, Riley concluded that the function of the mast cell is probably associated with the maintenance and repair of connective tissues.

Fig. 9.—Photomicrograph (reduced to 2/3 of mag. × 50) showing an 18-day healing fracture of the tibia in a young rat reared on a calcium-deficient diet for a period of 36 days. Note dark-staining mass of fibrocartilaginous callus in the lower left side of the picture; osteoid tissue in the periosteal callus without any mast cells on the upper left side, and endosteum and numerous mast cells lining the marrow cavity.



Experimental studies on mast cells in the past have dealt with cell counts in one or another location, e. g., (1) a membrane, such as mesentery, pleura, or synovia; (2) a perivascular connective tissue focus in tissue, such as skeletal muscle, heart, or visceral organs; (3) a complex tissue, such as bone marrow, or (4) a pathological tissue, such as a clinical or experimental tumor. The mast cell in cancellous bone appeared at first as if it might be a variation of either the perivascular or the marrow location. On closer inspection it was found in a pattern that was quite different from that of any of these locations. There were two other possibilities to consider about the position of mast cells in relation to bone tissue. The first was that mast cells may have formed in bone marrow and migrated toward and into the endosteum. This is based on the idea that mast cells possess motility, as some cytologists believe, and that they were formed in response to a change in the metabolism of the loose connective tissue of the bone marrow. The second possibility was that mast cells formed in the endosteum (defined as a layer of condensation of reticular cells) in response to alterations in the metabolism of bone tissue itself. Of the four elements that comprise the structure of bone tissue—cells, collagen, ground substance, and inorganic salts—the one that has been connected with the metabolism of the mast cell is ground substance. Uptake of radioactive sulfur in the granules of mast cells described by Asboe-Hansen [1] and Duthie and Barker [4] would tend to support such a hypothesis. On the basis of good experimental evidence, two substances, histamine and heparin, have been found to be present in mast cells.

In addition, from histochemical preparations, claims have been made for the existence of a number of other intracellular materials and enzymes: alkaline phosphatase, acid phosphatase, lipid, phospholipid, peroxide, cytochrome oxidase, lipase, glycogen, free iron, and unidentified proteins. Of these, the alkaline phosphatase content conceivably could have some relationship to the endosteal location of mast cells and the formation of collagen fibers in the bone matrix in calcium-deficient rats. This is, of course, merely speculation, and it is safe to conclude only that the relationship of histamine, heparin, sulfated hyaluronic acid precursors, alkaline phosphatase, and other substances to the local metabolism of connective tissue awaits further study.[1, 8, 9] An interesting suggestion about the function of the mast cell is that it may be a storage cell rather than a secretory cell, and that it is unused materials that are stored in its large cytoplasmic granules.

A controversial and important recent observation is the phenomenon of "explosion" of mast cells, in which the cell membrane is ruptured; the cytoplasmic granules are thus set free in the tissue spaces. Although it has not yet been generally accepted that cell explosion is physiological behavior, it seems to be a characteristic reaction of mast cells to chemical compounds and various agents classed as histamine liberators[2, 9]; no other connective tissue cells seem to be able to react in vitro in this way. It was not seen, in vivo, in our study during regression of mast cells from the bones of calcium-deficient rats after treatment with large doses of vitamin D. Instead, the mast cells gradually lost their granules and disappeared into the bone marrow when the rachitic metaphysis was revascularized and absorbed.

The changes in the cancellous bone and bone marrow described in this report must be regarded as the consequence of at least three factors: vitamin-D deficiency, inadequate calcium intake, and secondary hyperparathyroidism. In the presence of vitamin D, a diet as low as the 0.06% calcium in Shohl's Diet E might improve absorption of calcium and utilization enough to prevent florid rickets. In the absence of vitamin D, the total bone mass is reduced and osteoporosis develops in addition to rickets as a result of low calcium intake. This condition is quite different from ordinary rickets, in which the phosphorus content of the diet is low and calcium is normal. In osteoporotic rats reared on diets with insufficient supply of calcium the level of the serum calcium is low and the serum phosphorus is normal. This superimposes still another disorder of the skeleton. According to de Robertis and Stoerk and Carnes, low-calcium diets produce great hypertrophy of the parathyroid glands. Typical florid rickets produced in rats by means of a high calcium-low phosphorus diet causes slight hypertrophy of the parathyroids; the increase in the volume of the glands is relatively little and the number of osmiophilic cells, vacuoles, and other evidences of hyperactivity are pronounced only in rats on low-calcium diets. These observations indicate that secondary hyperparathyroidism was the basis of osteitis fibrosa observed in rats on Shohl's Diet E. Hyperparathyroidism and osteitis fibrosa caused by inadequate calcium intake in young rats favors formation of mast cells. Whether osteoporosis is possible without secondary hyperparathyroidism and whether osteoporosis alone may produce endosteal mast cells remains to be determined by further studies in experimental animals and clinical patients with various forms of bone disease.

Summary

Young rats weaned at 3 weeks of age to a diet low in calcium known as Shohl's Diet E produce mast cells in a specific location and in larger numbers than are ever seen under normal conditions anywhere in the body. The mast cells accumulate on the surface of, within, or under the endosteum when growth becomes arrested in these

animals at 7 to 10 weeks of age. This effect is characteristically seen in bone tissue and does not include a corresponding increase in population of mast cells in mesentery, perivascular connective tissue of muscle, or synovial membrane.

Treatment of the animals with large doses of vitamin D restores the normal processes of calcification and ossification and produces new bone and bone marrow. The mast cells bordering on the areas of repair become displaced or dispersed by proliferating osteoblasts and gradually disappear into the marrow. These show gradual thinning of their granules and reversion to a spindle-shaped form. Large doses of parathyroid injection U. S. P. produce osteitis fibrosa and do not diminish the number of mast cells or their granules.

Whether mast cells store histamine and heparin, a sulfated precursor of the ground substance of connective tissue, alkaline phosphatase, or any other material should be investigated further in the skeleton of calcium-deficient rats.

NOTE.—While this paper was in press, Dr. Richard H. Follis called our attention to an earlier description of "basophilic connective tissue cells near the trabeculae of bone" in rats fed on calcium-deficient diets. These cells, described and illustrated by Shipley and Park,[14] are certainly the mast cells described in the present paper, although they were not so identified.

Mead Johnson & Company, Evansville, Ind., donated the calciferol (900,000 I. U. vitamin D activity per cubic centimeter).

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Dual Morphologic Reactions of Rabbit Lymphocytes to X-Rays

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Previous studies [1] have led to the hypothesis that x-rays may produce different primary or immediate effects in different cells. More specifically the question was raised whether rabbit lymphocytes and amphibian ova react differently to irradiation. If x-rays have more than one primary effect in different cells, it may also have more than one effect in the same cell. This paper demonstrates that lymphocytes have two distinct morphologic reactions to x-rays.

Formation of intranuclear vacuoles is one response of lymphocytes to x-rays. The vacuoles have been demonstrated by dark-field [2] and phase microscopy of living cells, by time-lapse cinemicrography,[3] by freezing and drying techniques,[4] and by routine histologic methods.[5,6] The vacuoles were observed when the cells were irradiated in cellular suspensions,[2] in tissue culture,[6] and in the intact animal.[5,7] The intranuclear vacuoles have been reported in lymphocytes derived from thymus of rabbits,[3] from lymph nodes of rats,[6] and from the lymph of the thoracic duct of rats and dogs.[7]

Other morphologic changes in irradiated lymphocytes have been reported. Refractile granules were seen by Dickie and Hempelmann [8] in the cytoplasm of lymphocytes of persons chronically exposed to ionizing radiation. Ross, Furth, and Bigelow [7] found that lymphocytes from the thoracic duct of irradiated rats and dogs developed vacuoles in the cytoplasm as well as in the nucleus and that some lymphocytes were bi- or multinucleated. Bilobed nuclei were also observed by Ingram and Barnes.[9]

In the present work, the reactions other than intranuclear vacuoles are studied. These reactions were readily elicited in lymphocytes by use of large doses of x-rays.

Observations

Method.—Suspensions were prepared from the rabbit thymus by the methods described previously.[2] The cells were suspended in 50% rabbit serum. A drop of the suspension was placed between two cover glasses separated by a metal ring. The preparations were irradiated by a 100 kv. x-ray machine, 5 ma., 0.5 mm. equivalent Al filter. The half-value was 1.3 mm. Al, and the output was 670 r per minute. The slide preparations were incubated at 37 C and photographed by time-lapse cinemicrography. With this method, control, nonirradiated suspensions showed survival of nearly all of the lymphocytes for 8 hours and 50% or more of the cells remained normal after 24 hours of incubation. The effects of irradiation with 10,000 r on a group of lymphocytes are shown in Figures 1 to 6, which present prints made from one cinemicrographic film.

Observations.—Forty-three minutes after irradiation with 10,000 r, the lymphocytes had relatively large, round, prominent nuclei with thin nuclear walls and large chromatin masses (Fig. 1). The cytoplasm was sparse and inconspicuous and frequently could not be seen. The cells were surrounded by thin halos, which were an artifact produced by phase microscopy. All of the cells were round, and none had anterior pseudopods or a posterior tail. Nearly all of the cells showed the rhythmic, Brownian-like movement described previously.[1] In addition, some of the cells had an irregular rotating movement. The movements

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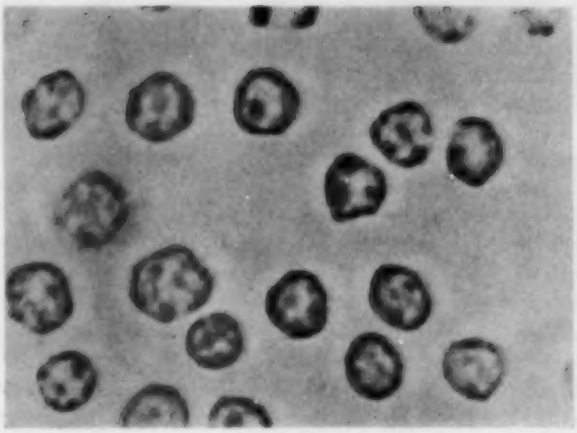

Fig. 1.—Rabbit lymphocytes irradiated with 10,000 r and incubated at 37 C for 43 minutes. The cells appear normal, with large prominent round nuclei and coarse chromatin granules. The print in this and in subsequent figures was made from a time-lapse cinemicrographic film. Reduced to 84% of mag. × 1750.

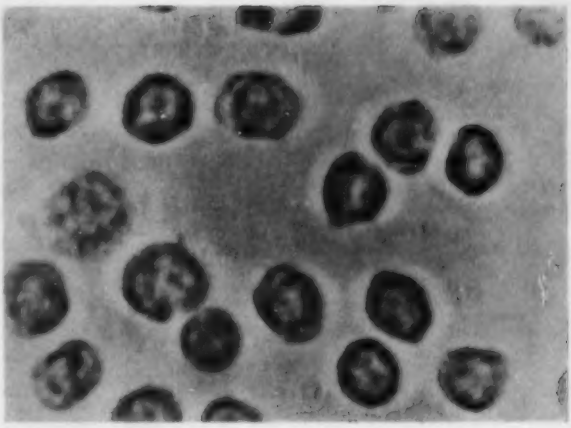

Fig. 2.—The print, made of two successive frames of the time lapse cinemicrographic film, shows the position of the cells at 43 minutes (as in Figure 1) and 6 seconds later. Nearly all the cells are viable and show oscillatory movement, as indicated by blurring and double contour.

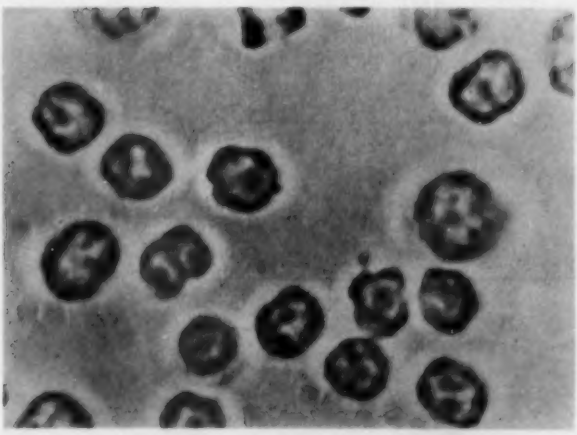

Fig. 3.—The lymphocytes of Figures 1 and 2, 61 minutes after irradiation. Many of the cells have irregularly shaped nuclei, and one cell has a bilobed nucleus.

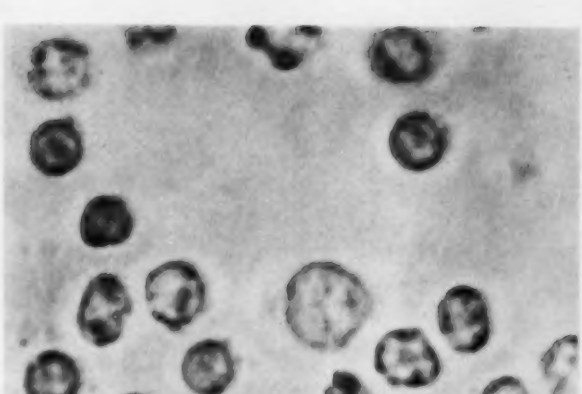

Fig. 4.—The cells of Figures 1 to 3 but 93 minutes after irradiation. Nearly all the nuclei have rounded up and show a slight shrinkage and blurring. A large cell has moved from its position in Figure 3 and has retained its large granular nucleus.

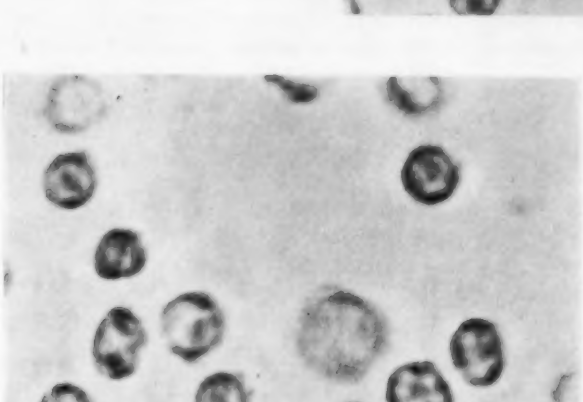

Fig. 5.—A print of four successive frames showing the cells of Figure 4 and the same cells 6, 12, and 18 seconds later. Most of the cells have shown no change of position. These immobile cells are dead. The large cell appears blurred and is still viable.

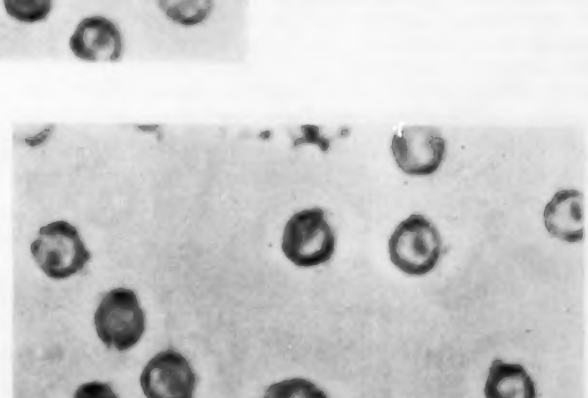

Fig. 6.—The cells of previous Figures 3.7 hours after irradiation. One cell is still viable with a large, normal nucleus. The other cells are dead and occupy more or less the same position as in Figure 4. The dead cells show further post-mortem change, as is indicated by the small, blurred nuclei.

are indicated in Figure 2, which is a composite print of two successive frames, starting from the frame shown in Figure 1. Nearly all the cells appear blurred and double in the Figure, the blurring being due to the oscillations of the cells. In view of the normal morphology and active oscillatory movement, the cells are considered to be viable and apparently normal in spite of the irradiation with 10,000 r and incubation at 37 C for 43 minutes.

One of the first signs of degenerative changes in the irradiated cells was the development of slight irregularities in the shape of the nucleus. Many of the nuclei developed one or more small, narrow indentations. The indentations were usually irregularly scattered over the surface of the nucleus, but occasionally they were symmetrical, so that the nucleus had a cloverleaf appearance. The narrow indentations sometimes deepened, with the gradual formation of two- or three-lobed nuclei; the lobes of the nuclei were usually equal in size, were oval with coarse granules, and apparently did not divide the nucleus completely. The cytoplasm of the irradiated cells also occasionally developed early degenerative changes with the formation of small vacuoles or thin cytoplasmic filaments.

After 61 minutes of incubation many cells had irregularly shaped nuclei with multiple narrow indentations (Fig. 3). One cell had a bilobed nucleus, and another cell, a cytoplasmic filament. In the projected film, the cells with the irregular nuclei had normal rhythmic oscillations. Most of the cells in the Figure were morphologically normal, and many cells never developed nuclear indentations or cytoplasmic vacuoles.

The next change in the irradiated cell was subtle and difficult to observe but was none the less important. On close observation of the projected film it was seen that, after about 70 minutes of incubation, one cell after another suddenly lost its rhythmic movement and became motionless. In some cases the sudden loss of oscillation was associated with a slight change in the optical focus of the cell. Apparently the cell had settled down on the slide. In addition, irregularly shaped or lobed nuclei suddenly rounded up, and the nucleoplasm became more transparent. There is reason to believe that these sudden changes—loss of oscillatory movements, change in the optical focus, and rounding up of the nucleus—are an indication of acute cell death; in other words, delayed "fixation." The term fixation is defined as death of the cell with only minimal immediate morphologic change.

Death by delayed fixation was seen to occur both for the morphologically normal cells and for the cells with indented or lobed nuclei. A few cells apparently died by fixation without immediate loss of rhythmic movements. The retained movements were sometimes seen to be transmitted from adjacent cells. The preparations used were not ideal for studying cessation of oscillations, since the field was crowded with cells. In most cells the time of death could be fairly definitely determined in the projected films. It should be pointed out that it has been found in this and other studies with other reagents that oscillations of a lymphocyte do not necessarily mean that the cell is viable, although the sudden loss of oscillations is an indication of death by fixation.

Figure 4 shows the cells 93 minutes after irradiation. The cells seem to be morphologically normal and, in fact, have lost some of the early degenerative changes seen in Figure 3. An indication of a functional change in the cells can be seen in Figure 5, which is a composite print of four successive frames, starting from the one shown in Figure 4. The cells in Figure 5 do not show any change in position during the 16 seconds between the first and the last picture. These immobile cells were considered dead. Only one cell shows blurring or movement, and this cell is seen to be viable in the projected film.

The lymphocytes that died by fixation underwent further changes which may be considered as postmortem change or autolysis. The earliest signs of autolysis can be

seen in Figure 4, where the cells had been dead for 5 to 20 minutes. The nuclei have developed slight haziness and have lost their crisp appearance, as seen in the viable cells of Figure 1. This slight blurring in the structure of the nucleus is difficult to recognize and has to be differentiated from artifacts introduced by photography or microscopy. With further autolysis, the nuclei decreased in size and became darker, so that it was difficult to make out the chromatin masses. While the nuclei underwent shrinkage, blurring, and darkening, the cytoplasm became increased in amount, edematous, and clear. The cell wall appeared as a thin line enclosing the clear invisible cytoplasm. Some of these autolytic changes in the nuclei are seen in Figure 6, which shows cells 3.7 hours after irradiation. After 20 hours of incubation the irradiated cells killed by delayed fixation were small and clear and had a thin, dark reticular network.

In Figure 6, one cell is still viable 3.7 hours after irradiation with 10,000 r. This cell is seen to be a large lymphocyte. In other experiments it has appeared that the large lymphocyte is slightly less radiosensitive than the small cell and succumbs somewhat later to the x-ray treatment.

The cinemicrographic film which was used to prepare the prints of Figures 1 to 6 was studied in detail and each cell followed up. The findings are summarized in the accompanying Table. Fifteen cells were observed to die, and all of the deaths were by fixation. The cells died 1.2 to 3.0 hours after irradiation. The medium survival time of these 15 cells was 1.5 hours.

The reactions of cells from the rabbit thymus to 1000 r of x-rays have been reported previously in detail.[3] It may be useful to review the findings briefly and to compare them with the present observations with 10,000 r. The early degenerative changes after 1000 r occurred usually two or more hours after irradiation and consisted of small, single or multiple intranuclear vacuoles, followed by a slight irregularity in the shape of the nucleus. These changes were probably invariable antecedents to the more drastic changes that occurred subsequently. On the other hand, the early changes after 10,000 r, namely, small cytoplasmic vacuoles and irregular nuclear shape, apparently occurred in relatively few irradiated cells, and most cells died by fixation without any preliminary degenerative change.

The rabbit lymphocytes developed, in a few hours after irradiation with 1000 r, extensive lobulations of the nucleus. These rapidly changing lobulations were quite different from the gradual development of the lobed nucleus after 10,000 r. The primary

Summary of Cell Death as Observed in Cinemicrographic Films of Lymphocytes Irradiated with 1000 to 10,000 r and Incubated at 37 C

	Dosage of X-Rays			
	10,000 r	4,000 r	2,000 r	1,000 r
Cells dead at onset of photography				
Number	1	0	1	1
Cells killed by fixation				
Number	13	14	8	0
Time of death*				
Range	1.2-3.0	0.6-2.3	0.5-2.3	--
Median	1.5	1.0	0.9	--
Cells killed by intranuclear vacuolation				
Number	0	4	18	20
Time of death*				
Range	--	2.0-4.5	1.4-3.8	1.3-8.3
Median	--	2.4	3.0	5.5
Cells killed by atypical reaction				
Number	0	1	0	0
Time of death*	--	0.6	--	--
Cells viable at end of film				
Number	1	1	0	3
Time*	4.0	5.2	5.0	8.5

* Time in hours.

change in the 1000-r-irradiated cell seemed to be not the lobulations but the intranuclear vacuoles, which grew rapidly and pushed the chromatin peripherally until one or more chromatin rings were formed. The rings soon ruptured, and the chromatin contracted to form a small structureless, pyknotic spherical mass.

The pyknotic nucleus had a superficial resemblance to the fixed nucleus with early or late autolysis. In the projected film there was no problem in differentiating the two; the pyknotic nucleus followed the prominent intranuclear vacuoles, while the nucleus undergoing autolysis resulted from a slow, gradual loss of morphologic detail in a quiescent cell. Even in a single print it was possible to recognize the pyknotic nucleus, with its small, dark, completely homogeneous mass, which was frequently associated with similar smaller fragments in the same cell. The nucleus with early autolysis also appeared small and dark, but it was usually possible to make out chromatin masses. Ultimately, under the proper conditions, both types of nuclei underwent karyolysis, and then it was not possible to make a postmortem diagnosis of the cause of death of the cell.

One cinemicrographic film showing the effects produced by 1000 r was reviewed and summarized in the Table. In this film, 20 cells were seen to undergo death, and all the deaths were by vacuolation and lobulations. No deaths were by delayed fixation, such as occurred after 10,000 r. The observed time of death ranged from 1.3 to 8.3 hours. Death by vacuolation after 1000 r occurred later than death by delayed fixation after 10,000 r.

To summarize, the irradiation with 1000 r produced, after a latent period of several hours, rapid and dramatic changes of intranuclear vacuolation and lobulation. These rapid changes led to the death of the cell in about 20 minutes, with the development of a pyknotic fragmented nucleus. In contrast, 10,000 r caused, after a latent period of about one hour, acute death or delayed fixation of the cell.

Other cinemicrographic films were prepared and analyzed to determine the effect of the intermediate doses of 2000 and 4000 r (Table). With these doses, cells died by delayed fixation, by vacuolation and lobulation, and by an atypical reaction. The observed numbers of cells were small, and the findings are considered only semiquantitative. The Table shows that 4000 r killed 14 cells by fixation in 0.6 to 2.3 hours after irradiation and 4 cells by vacuolation in 2.0 to 4.5 hours. With 2000 r 18 cells died by vacuolation and 8 by delayed fixation. It is definite that the intermediate doses of 2000 and 4000 r caused both types of death and that in general delayed fixation killed the cells earlier than vacuolation.

As indicated previously, a few cells showed an atypical reaction. The morphologic changes in these cells were quite variable. One cell showed lobulation involving the cytoplasm, while the nucleus died by fixation. Another cell showed vacuolation of the nucleus with formation of a chromatin ring but no lobulation of the cell or nucleus. A third cell started lobulation and suddenly died by fixation. These atypical reactions suggested that the cells had suffered various combinations of both types of reactions.

Comment

The present study shows that the rabbit lymphocyte has two distinct types of morphologic reaction to x-rays. For purposes of classification and description these reactions may be called Type V and Type F. One type of reaction was elicited by irradiation with 50 to 1000 r and produced intranuclear vacuoles, lobulations of the cell and nucleus, and, finally, a pyknotic, fragmented nucleus. This reaction, characterized particularly by intranuclear vacuoles and low doses of x-rays, is termed Reaction V. A second type of reaction of lymphocytes was observed after irradiation with 10,000 r. The large doses of x-rays produced, after

a short latent period, minor degenerative changes in some of the viable cells, such as cytoplasmic vacuoles and filaments, indentations of the nuclear wall, and bi- or trilobed nuclei. The more important effect of the irradiation was delayed fixation, i. e., acute death after a short latent period. The death of the cell was indicated by loss of oscillatory movement, change in optical focus of the cell, rounding up of the nucleus, and increased transparency of the nucleoplasm. The dead cells later underwent postmortem autolytic changes. This Reaction F is characterized by high doses of x-rays and delayed fixation of the cell.

It has been seen that Reaction V occurred after 1000 r and Reaction F after 10,000 r. After intermediate doses, the lymphocytes died either by vacuolation or by fixation, and a few cells showed a combined type of reaction. It would seem that both types of reaction occurred in the same cell, although one or the other reaction usually predominated.

The question arises whether Reactions V and F occur in other cells besides lymphocytes. At present there are no absolute criteria for classifying the diverse cytologic changes reported by different investigators. As a tentative plan for classification, we may define all changes in nondividing cells produced by irradiation with 1000 r or less as Reaction V and with 2000 to 10,000 r as Reaction F. In the absence of a comparable irradiation dose, the types of morphologic change may aid in classification.

Warren et al.[4] showed by the freezing and drying technique that radiosensitive cells, such as the epithelial cells of intestinal crypts, developed on irradiation large intranuclear vacuoles, which grew, broke the nuclear wall, and killed the cell. These vacuoles seemed identical with those observed in lymphocytes. Even hepatic cells developed vacuoles after irradiation, but in these radioresistant cells the vacuoles remained small and were extruded from the nucleus. By careful histologic technique, Warthin [10] demonstrated small vacuoles in the renal cells of irradiated mice. Both the small vacuoles of radioresistant cells and the large lethal vacuoles of radiosensitive cells are classified as Reaction V according to the criteria used. It may be concluded that Reaction V occurs in many types of cells, both radiosensitive and radioresistant.

Stroud and Brues [11] irradiated chick fibroblasts in tissue culture with tritium. Low doses of radiation caused interference with mitotic division. High doses, on the other hand, produced sudden death of resting cells with shrinkage of the nucleus. Unfortunately, the dose given with tritium cannot be compared directly with the 10,000 r used in this study. The cytologic changes produced by high doses may be classified as Reaction F.

Puck and Marcus [12] irradiated HeLa cells with 50 to 10,000 r. At the lower doses, the cells showed early or delayed interference with mitotic division. At high doses (10,000 r) most cells did not divide but ultimately disappeared. The morphologic changes leading to lysis are not described but probably represent Reaction F with autolysis.

Duryee [13] observed changes in the nucleoli and chromosomes of resting ova of amphibia after irradiation in vitro with 2000 to 10,000 r. The changes may be attributed to radiation effect F, since the doses were relatively large and the cells were not in mitotic division.

The two types of reaction of lymphocytes to x-rays raise the question whether irradiation causes two types of cellular injuries. The F reaction seemed implicated in the death of the nondividing fibroblast,[11] the HeLa cell,[12] and the amphibian ova,[13] as well as of the radiosensitive lymphocyte. The F reaction is apparently more widespread among cells than the V reaction. Furthermore, it has been seen in the present study that the same dose of x-rays (between 1000 and 10,000 r) produced both types of death and that occasionally both reactions occurred in the same cell. These data would suggest that the two types of reactions are

distinct and are dependent upon different types of cellular injury. The primary injuries would lead to secondary reactions and would ultimately kill the cells either by delayed fixation or by vacuolation or by both. Although the V reaction was best demonstrated by use of 1000 r and the F reaction by 10,000 r, it would be necessary to assume that any dose of x-rays produced both types of cellular injury and that the type of death would depend on the relative rates of the secondary reactions following the two cellular injuries.

As pointed out in a previous publication,[3] diverse evidence indicates that vacuolation may be attributed to alterations in the metabolism of deoxyribonucleic acid. Duryee's data[13] on the death of amphibian ova, which has been classified as Type F, may be helpful in understanding the primary change in this reaction. Duryee was able to induce the nuclear changes in nonirradiated ova by injection of irradiated cytoplasm. This finding suggests that Reaction F, or death by fixation, may be due to a primary change in the cytoplasm.

Summary and Conclusion

Rabbit lymphocytes were irradiated in vitro, incubated at 37 C, and studied by time-lapse cinemicrography. After 10,000 r, some of the cells developed early degenerative changes, including irregularities in the shape of the nuclei and clover-leaf and bilobed nuclei. In one to three hours all the cells were seen to undergo death, as indicated by loss of oscillatory and rotational movements, rounding up of the nucleus, and increased transparency of the nucleoplasm. The cells, however, retained many of their morphologic characteristics. This type of death was called delayed fixation. After irradiation with 1000 r, the lymphocytes died by intranuclear vacuolation, lobulation, and pyknosis. With intermediate doses (2000 and 4000 r), some cells died by delayed fixation, some by intranuclear vacuolation, and a few by a combined process. The two types of death suggest that x-rays produce two distinct types of cellular injury.

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Paradoxical Mucorthrombosis in Thrombocytopenic Purpura

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Introduction

Mucormycosis is now a well-established disease entity. The term applies to infections caused by fungi of the order Mucorales, including the genera Mucor and Rhizopus. Baker has recently reported six new cases and one previously abstracted finding of pulmonary mucormycosis.[1,2] He has also reviewed reports from the older German literature.[3-7] Lloyd, Sexton, and Hertig reported pulmonary mucormycosis complicating pregnancy.[8] Twelve instances of cerebral mucormycosis have been reported.[4,9-16] All of the above cases were established by autopsy findings, but additional infections by fungi of the order Mucorales have been reported on the basis of examination and culture of material from sputum,[17,18,19] excised lung tissue,[20] and the nasopharynx.[21]

When mucormycosis has been implicated in the death of the patient, widespread thrombosis of the vessels of the lungs and/or brain has been found at autopsy. In the recent case reported by Zimmerman the patient's death was attributed to a "Mucor-induced coronary venous thrombus," but similar thrombi were also present in the pulmonary and renal vessels.[22]

We wish to report a case of pulmonary mucormycosis which was paradoxical in that widespread pulmonary thrombosis occurred in the presence of long-standing severe thrombocytopenic purpura.

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Illustrations were prepared by Mr. H. Paul Newman, Chief, Medical Illustration Laboratory, Veterans Administration Hospital.

Report of a Case

A 24-year-old white man was admitted to the hospital after a syncopal attack Jan. 17, 1955. He had been discharged from the Navy in October, 1954, and had remained in good health until he developed a sore throat one month prior to admission. In rapid succession, he suffered with pustular eruptions of the skin, enlargement of cervical and axillary nodes, bleeding gums, and severe progressive weakness and fatigue.

The past history was not revealing, but it is interesting that he served a total of eight to nine months in the Bikini Island area between 1951 and 1954 and was present at a total of seven atomic explosions. There was no family history of cancer or leukemia.

Physical examination revealed a weak, but alert, young man with enlarged lymph nodes in the cervical, axillary, tonsillar, and inguinal regions. Ecchymoses of the legs and bleeding from the gums were noted. The spleen was palpable 3 cm. below the left costal margin.

Chest x-ray revealed abnormal rounded masses in both hilar regions. Initial laboratory data included hematocrit of 19%; white blood cell count, 98,000/cu.mm.; platelet count, 70,000/cu.mm.; sedimentation rate, 74 mm/hr.; reticulocyte count, 0.4%; and a differential count which included approximately 80% "blasts." Sternal-marrow aspirate was hypercellular and contained predominantly "blast" forms, with enough differentiation toward early myelocytes to establish the diagnosis of acute myelogenous leukemia. Megakaryocytes were not seen in this marrow aspirate. Urinalysis and blood chemistry determinations were within normal limits.

He was treated for two weeks with cortisone, transfusions, and 6-mercaptopurine, with rapid improvement clinically and more gradual improvement in the blood picture. Within the two weeks his white cell count reached 2400/cu.mm. and remained between this level and 10,000/cu.mm. for three months. The platelet count averaged 30,000/cu.mm. during this same period without marked variation, and megakaryocytes were rarely encountered in the smears and sections of sternal

marrow. At this time the previously enlarged nodes and spleen were not palpable.

The first remission, however, was short-lived, and he returned to the hospital on March 15, 1955, with acute maxillary sinusitis. He was treated vigorously with antibiotics, 6-mercaptopurine, transfusions, and cortisone. There was little change until his terminal hospital admission, on July 18, 1955. The presenting complaints at that time were weakness and epistaxis. His white blood cell count had risen to 139,000/cu.mm., and the platelet count was only 9000/cu.mm. Because of his downhill course, A-methopterin (4-amino-10-methylpteroylglutamic acid) 2.5 mg. daily was begun on Sept. 7.

During the last three weeks of life an interesting series of findings developed in the chest. On Sept. 2 he complained of left pleuritic pain. The chest x-ray three days later revealed "pneumonic infiltration of the anterior subapical segments of the left upper lobe." Two weeks later there were consolidation of the entire left upper lobe and patchy infiltration of the remaining lung fields. A leukemic infiltration with bronchial obstruction in the left upper lobe was considered more likely than bacterial pneumonia by the radiologist. X-ray follow-up just prior to death revealed no further change.

During this terminal three-week period he received 9 units of whole blood. Despite this, his hematocrit averaged 20% and his white blood cell count remained close to 2500/cu.mm. During the last two months of hospitalization the highest platelet count recorded was 10,000/cu.mm. and counts ranged as low as 3000/cu.mm. Sputum smears for acid-fast bacilli were negative. Samples were not obtained for culture of acid-fast bacilli, fungi, or predominating organisms.

Ecchymoses and petechiae became worse during the last week of life. At the same time he began coughing up bloody sputum, which increased to the extent of gross hemoptysis five days prior to death. Left shoulder pain, cough, and hemoptysis persisted, and dyspnea increased continuously. He became moribund and died on Sept. 25, 1955.

Autopsy Findings

A complete autopsy was performed 16 hours after death. Throughout the skin and serous surfaces were widespread ecchymoses and petechiae, and there were moderate pleural and pericardial sanguineous effusions. The heart weighed 450 gm. and revealed hypertrophy of both ventricles without dilatation of the chambers. Distal to the pulmonic valve ring a large, grayish-pink, firm thrombus filled the main pulmonary artery except for a slit-like channel leading to the right branch (Fig. 1). The left pulmonary artery was

Fig. 1.—Pulmonary valve and artery showing proximal portion of mucorthrombus.

entirely plugged by an adherent antemortem thrombus. When the left atrium was opened, a similar thrombus was found filling the left superior pulmonary vein and occupying the adjacent portion of the atrium, but there was no obstruction to the flow of blood from the other pulmonary veins.

The lungs weighed 2250 gm. The left lung, which was the heavier, revealed widespread arterial and venous thrombosis with infarction of the entire upper lobe and large segments of the lower lobe. The right lung was congested, but no thrombosis or infarction was found.

The liver weighed 1850 gm. and the architecture was preserved. The firm, red spleen, without prominent follicles, weighed 750 gm. but contained no nodules or focal changes. The bone marrow was red in color, without gross change.

Microscopically, there were leukemic infiltrates in the bone marrow, spleen, renal pelvis, and testes. These consisted primarily of "blast" forms, but there was occasional differentiation toward the myelogenous series. In the bone marrow erythropoiesis was strikingly diminished and megakaryocytes were quite rare.

Within the lungs there was abundant evidence of chronic passive congestion with hemorrhage, interstitial scarring, proliferation of young fibroblasts, and pigment-laden macrophages. The left lung revealed coagulation necrosis with hemorrhage and chronic and acute inflammatory cells. The thrombi within the pulmonary vessels consisted of tangled masses of hyphae with granular eosinophilic debris and occasionally enmeshed erythrocytes (Fig. 2). Coagulation necrosis of the arterial walls and surround-

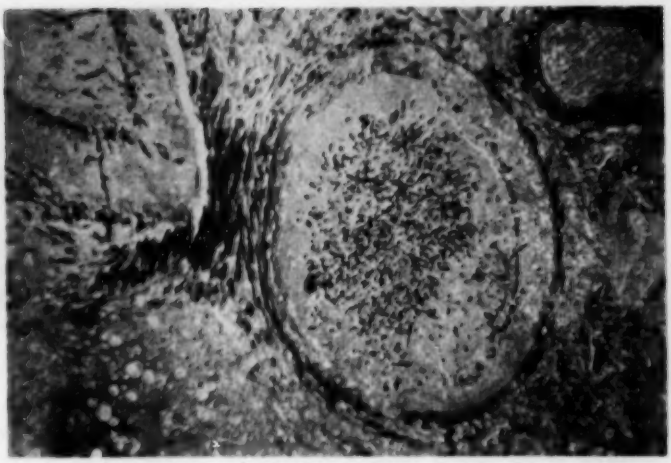

Fig. 2.—Low-power view of small branches of pulmonary artery filled by thrombi containing numerous hyphae. Gomori methenamine-silver nitrate stain; reduced to 62% of mag. × 135.

ing tissue was frequent, and mycelial elements were found infiltrating the walls of vessels and in the adjacent tissue. Large mononuclear leukocytes surrounded the mycelia in the perivascular zones. Rare hyphae were seen within the bronchi.

The morphology of the fungus was defined most sharply by using Gomori's methenamine silver nitrate technique, as described by Grocott.[23,24] The bulk of the thrombus was composed of a branching, coenocytic mycelium varying from 3μ to 13μ in width, compatible with the morphology of the Mucorales (Fig. 3). An occasional structure was seen which was interpreted as an abortive sporangium (Fig. 4). Borne terminally upon a short mycelial stalk, the sporangium measured 25μ by 13μ and showed numerous small inclusions or spores. Unfortunately, no tissue was obtained for culture prior to fixation of the tissues, so that differentiation between Mucor and Rhizopus could not be made. Dr. R. D. Baker, who reviewed the tissue sections, agreed that the organism undoubtedly belongs in one of these two genera.[25]

Comment

This case is of interest both as a clinical and as a pathologic diagnostic problem. The

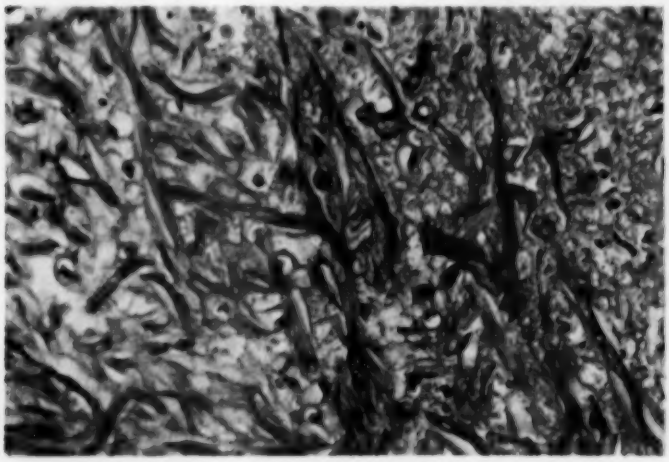

Fig. 3.—High-power view of branching nonseptate hyphae in pulmonary thrombus. Hematoxylin and eosin; reduced to 62% of mag. × 400.

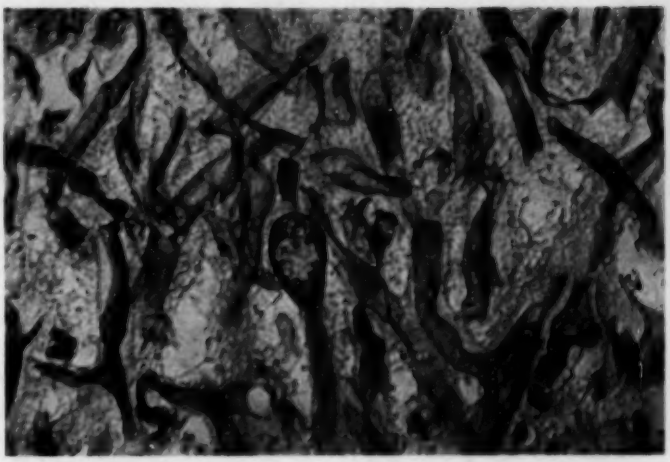

Fig. 4.—High-power view of hyphae with one terminally borne sporangium containing spores. Gomori methenamine-silver nitrate stain; reduced to 62% of mag. × 1000.

hematological consultants who saw the patient felt that ordinary thrombosis could not occur in the presence of the sustained platelet counts below 10,000/cu.mm. This led all observers away from the diagnosis of pulmonary thrombosis and infarction, which most nearly fitted the clinical picture. The radiologist also was reluctant to make a diagnosis of pulmonary infarction. At autopsy the pallor of the thrombi was impressive, but even this clue did not suggest the true etiology. For this reason, cultures were not made, and it is interesting that only in rare cases has the autopsy diagnosis of mucormycosis been proved by culture.[6,15]

The routine hematoxylin-eosin sections were adequate to make a diagnosis of fungus infection, and the order Mucorales was suspected prior to special staining because of the nonseptate hyphae. The mycelium failed to stain selectively using the Hotchkiss-McManus modification of the PAS stain, or the Gridley fungus stain, but the Gomori technique yielded excellent differentiation. Control slides of Coccidioides were well stained by all techniques.

Our case is quite typical in illustrating the pathogenesis and distribution of the fungus in pulmonary mucormycosis. The predominant masses of fungi lay within vessels and their walls, with resultant thrombus formation. Occasional hyphae were found within bronchi, but no luxurious growth was noted therein. Presumably, the organism enters the lungs via the bronchial tree and then invades the adjacent tissue prior to widespread proliferation in vessels. The latter seem to be the favored site of growth. The characteristic polymorphonuclear leukocytic reaction may have been limited in this case by the low white blood cell count. Large mononuclear cells dominated the reaction to the fungus in noninfarcted areas of the lung parenchyma. Such macrophages, and even giant cells, have been described previously in the reactive zones.[1]

Diabetes mellitus appears to be a common predisposing factor to mucormycosis in that 8 of the 12 cerebral cases[9-12,15] and 4 of the 10 recent pulmonary cases[1,2,8] occurred in diabetics. In most other cases, as in our case, severe debilitating disease, notably malignancy, was present. Baker, in his review, cites only one case without known predisposing factors. He emphasizes the predisposing role of antibiotics, which, by suppressing the growth of normal bacterial flora, permit normally saprophytic fungi to invade living tissue. He also suggests the possibility that cortisone, corticotropin, and antileukemic drugs favor the invasion of the fungus.[1] This view is particularly interesting because our patient was treated with cortisone, 6-mercaptopurine, and A-methopterin. His debility and anti-

biotic therapy, however, must be given more significance in evaluating the role of possible predisposing factors until more is known of the relationship between mucormycosis and antileukemic drugs.

It is our feeling that the patient's death was directly attributable to pulmonary mucormycosis, even though another invariably fatal disease was present.

No specific treatment for mucormycosis is known, and systemic, pulmonary, or cerebral mucormycosis has proved fatal in all reported cases.

Summary

A case of fatal pulmonary mucormycosis complicating acute myelogenous leukemia in a 24-year-old white man is reported. The case is of interest because of the rarity of the complication and the diagnostic problem which it presents. The thrombosis of the pulmonary vessels is paradoxical in that it occurred while the platelet counts ranged below 10,000/cu.mm. A brief review of previously reported cases of fatal pulmonary and cerebral mucormycosis is included, and the possible predisposing factors which have been suggested in these reports are discussed in relation to this case.

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Massive Calcinosiderotic Splenomegaly Simulating Portal Cirrhosis

Report of Two Cases and Review of the Literature

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Calcinosiderosis of the spleen is characterized by the presence of small rusty nodules produced by the deposit of iron and calcium. These foci have been called "tobacco nodules" and Gandy-Gamna bodies. They have been observed in cases of hepatic cirrhosis,[1-3] chronic splenic engorgement,[3-5] chronic thrombotic occlusion,[6] degenerative diseases of the splenic artery,[7] hemolytic icterus,[2,6] endocarditis,[3] and Graves' disease.[3] Only infrequently have they been associated with a splenomegaly of primary major clinical importance. Simonds,[8] in 1908, and Sprunt,[1] in 1911, each reported one case of massive calcinosiderotic splenomegaly. Symmers and his co-workers,[9] in 1919, recorded six cases observed at Bellevue Hospital. In 1948, he[10] mentioned three other cases seen at autopsy: two at Bellevue Hospital and one at Goldwater Memorial Hospital. Brackertz'[5] report of a case appeared in 1932. Fiessinger and his associates[11] reported one case under the term splenic reticulosis. This case had an exceptional neoformation of fibroblasts. In a study of anemias associated with splenomegaly, Stengel[12] included a case which may possibly fall in the same category. He did not mention the presence of calcium, but the spleen weighed 950 gm. and the illustration shows a lesion similar to those of the published cases and of our own. He mentioned encountering in his experience only one similar case. The purpose of the present communication is to report two cases of massive calcinosiderotic splenomegaly, review the literature, discuss the pathogenesis and the etiology, and present their relation to the current concepts of the hemodynamics of the portal system.

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Report of Cases

CASE 1.—The patient, a 32-year-old Puerto Rican man, was admitted to New York City Hospital on Nov. 26, 1947, because of marked weakness for one month and some loss of weight. The onset of the current illness was four years previously with abdominal swelling, which subsided spontaneously. Two years later he entered a hospital in Puerto Rico for recurrence of his abdominal enlargement; paracentesis was performed twice. A mass was palpable at this time in the left upper quadrant. He noted that the stools were yellow and on one occasion contained fresh blood. Subsequently he discovered a slowly developing jaundice. One year later there developed dribbling, oliguria, frequency, and a cloudy urine. These symptoms persisted up to his current hospitalization and were unassociated with dysuria and burning.

The only pertinent data in the past history were a "worms" infestation years before and the drinking of 2 to 4 qt. of beer nightly for five years prior to the first paracentesis. In the last two years he had lost 10 lb. (4.5 kg.) weight.

On admission, physical examination disclosed a chronically ill, emaciated, deeply jaundiced man lying quietly in bed. The most impressive finding was an enormously enlarged spleen, firm, regular, nontender, and reaching 10 fingerbreadths below the costal margin. There was a questionable fluid wave. The liver was not palpable. The lips were cyanotic; there was slight arterial pulsation in the supraclavicular fossae, and the neck veins were not distended. A soft blowing systolic murmur was audible at the apex. The lungs were clear. Rectal examination revealed fresh blood and light-colored feces on the examining finger. Hemorrhoids were

absent, and the prostate appeared normal. Spider angiomata and superficial lymphadenopathy were absent. The temperature was 98 F; the pulse rate 84 and respirations 22, per minute. The blood pressure ranged between 85 and 95 mm. Hg systolic and 50 and 60 mm. Hg diastolic.

The hematologic examination showed a red cell count of 4,800,000, hemoglobin 7.2 gm. per 100 cc., and white cells 3400, with 70% neutrophils and 30% lymphocytes and a normal sedimentation rate. Blood chemical examinations revealed normal alkaline phosphatase values of 4.7 to 5.0 Bodansky units, an elevated icterus index of 13 to 28 units, and a prolonged prothrombin time. Urinary urobilinogen was present in dilutions of 1:30 and 1:40. The stools were negative for parasites on several occasions. The blood Wassermann reaction was negative.

An extensive network of varices, confirmed by esophagoscopy and roentgenography, extended 20 cm. from the incisors to the cardia of the stomach. Intrinsic organic disease of the stomach, duodenum, and colon appeared to be absent on roentgenologic study, but there was an elevated left diaphragm and an enlarged spleen displacing the transverse colon.

The most striking clinical features which this patient exhibited were a severe, progressive anemia, cachexia, and splenomegaly. The erythrocytes fell to 3,280,000 and the hemoglobin to 6.5 gm. per 100 cc. The red cells showed anisocytosis, hypochromia, and polychromasia; occasionally there were a few normoblasts. The white cells fell to 2900 and showed a shift to the left, with myelocytes and a rare promyelocyte present. Bone marrow studies revealed a marked erythroid hyperplasia, with a shift to the left and both erythroblasts and megaloblasts present in considerable numbers. There were no significant changes in the granulocytic series.

During the ninth week, an exploratory laparotomy was performed. On opening the abdomen, such extensive vascularized perisplenic adhesions were encountered that bleeding became a major problem and all further surgical measures were abandoned. The temperature rose to 106 F immediately following operation but dropped to normal within three days. On the second day he became distended but responded fairly well to therapy. On the 9th and 10th postoperative days he suddenly had massive hematemesis and severe cramp-like epigastric pains. Death occurred on the 11th postoperative day, from terminal uncontrollable hematemesis.

Autopsy (performed six and one-half hours after death).—Only the pertinent findings are abstracted.

The body is that of an extremely emaciated white man appearing the stated age of 32 and showing marked jaundice of the skin and sclerae. The abdomen is distended, and has a fluid wave and a healing surgical incision in the left upper quadrant. There is scanty subcutaneous tissue.

The peritoneal cavity contains 2 liters of slightly blood-tinged clear fluid. The omentum is lightly adherent to the surgical incision and tightly adherent to the spleen.

The spleen, liver, diaphragm, and retroperitoneal tissues of the upper abdomen are fused together by dense, thick fibrosis. The fibrotic tissue surrounds the vessels at the splenic and hepatic hili, spreads over the undersurface of the liver and gall bladder like sugar icing, and binds the adjacent edges of liver and spleen together. The portal system has widespread organized thrombosis. The splenic vein and the portal vein to a point 2 cm. from the hilus of the liver are completely occluded. The thrombus extends for 3 cm. along one side of the superior mesenteric vein; here it does not produce complete occlusion. The oldest portion of the thrombus is at the splenic hilus. The portal vein is very large and greatly thickened. The hepatic, splenic, and mesenteric arteries appear normal.

The spleen fills the left upper quadrant, reaches the midline, extends slightly below the umbilical level, and weighs 1800 gm. The normal shape is retained; the consistence is firm. The cut surface is firm and sprinkled with discrete, well-demarcated, golden-brown foci 5 mm. or less in size and lying 1-2 cm. apart. The pulp is light reddish. The follicles are obscure.

The liver weighs 1500 gm. The edge lies at the costal margin. The dome is adherent to the diaphragm. On section, a pipestem cirrhotic process is seen to extend for a limited distance into the organ from the hilus. The deeper portions of the liver are soft, friable, and free of fibrosis. The portal venous radicles are dilated, and many tightly adherent mural thrombi are present; none is completely occlusive.

The esophagus has varices throughout its entire extent. There is an erosion at the junction of the middle and lower thirds. The stomach is distended with 1500 cc. of clotted blood. About an equal amount is present in the intestine. Sigmoidal, rectal, and hemorrhoidal veins are greatly dilated.

Microscopy.—Spleen: The trabeculae are thick and fibrosed and show lesions of varying severity. In the trabeculae having both artery and vein, the mildest lesion is that of dilation of vessels. Others have an inflammatory infiltrate of lymphoid, monocytic, and plasmocytic cells, most marked around the vein which appears compressed; hemorrhage is absent. Others have a diffuse in-

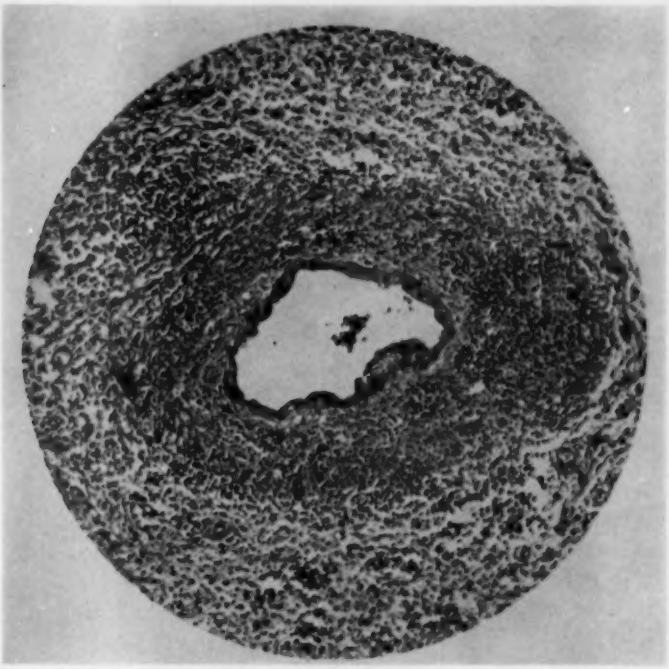

Fig. 1 (Case 1).—Early trabecular lesion showing dilated vein and diffuse recent hemorrhage. These lesions were more abundant in Case 1 than in Case 2. × 113.

filtrate and recent periphlebitic hemorrhage. Diffuse hemorrhage is sometimes present. In these trabeculae the veins are unidentifiable or are compressed. Regressive changes are present with the inflammatory process and consist of hyalinization of connective tissue, degeneration of elastic fibers, and deposition of hemosiderin and calcium, accompanied by a peripheral zone of recent hemorrhage. Both pigments are found throughout the trabeculae. The hemosiderin appears in two forms—one as granular, refractive, and golden brown; the other as crystalline, refractive, and yellow. Both give a positive Prussian-blue reaction. The calcium stains blue with hematoxylin and is soluble in acid alcohol without the evolution of gas. The calcium occurs as coarse masses at the periphery and as granular masses and coarse bands on the hyalinized connective tissue and degenerating elastica. As the artery passes through the periphery, it is surrounded by a collar of hemosiderin. Only in the heavily pigmented foci do both veins and arteries have hemosiderin collars. The trabeculae devoid of arteries have the same types of lesions. Peripheral zones of recent hemorrhage are prominent. Eggs of Schistosoma are not found.

The pulp has a diffuse reticular hyperplasia. In many areas, there are numerous hyperplastic reticular cells, some of which contain a fine granular nonrefractive brown pigment which fails to give an iron reaction with the Prussian-blue stain. Small foci of myeloid cells are present in scanty numbers.

The follicles are small and inconspicuous. A few have central hyalinization. The central arteries appear normal.

The capsule is thick, fibrotic, and hyaline. There are scattered foci of plasmocytes, eosinophils, and fibroblasts. Eggs are not found. The surface is covered with fibrin.

Liver: The cirrhotic process is mainly concentrated near the hilus and the inferior

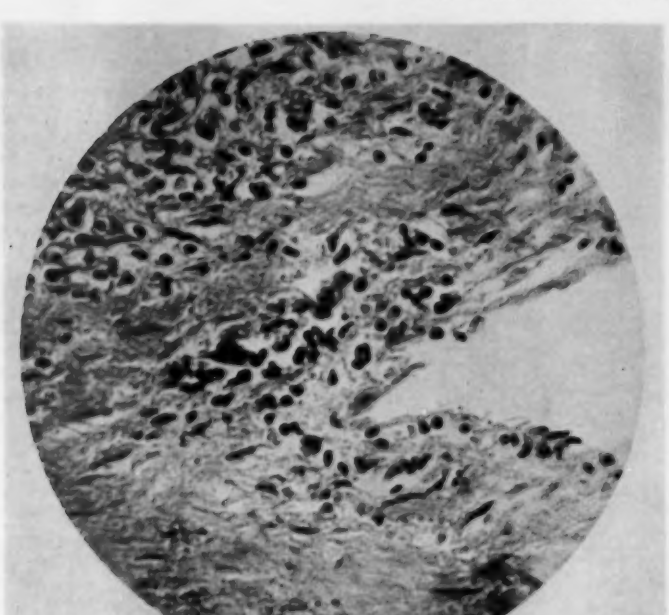

Fig. 2 (Case 1).—Inflammatory lesion of trabecular vein. Similar lesions were not found in Case 2. × 500.

surface. Portal fibrosis is severe. The bile ducts are proliferating, and some contain bile casts. The heavy inflammatory reaction is chiefly of lymphoid and plasma cells. The larger veins are dilated; many small veins have young mural thrombi. At the hilus there are a few Schistosoma eggs. A few ova are present at the advancing periphery of the cirrhosis.

The noncirrhotic parts of the liver have intralobular granulomatous foci with lymphoid, plasmocytic, and eosinophilic cells and giant cells phagocytizing Schistosoma eggs.

The perihepatic tissues show marked scarring and extensive inflammatory reaction. Ova are not seen in the dense hyaline diaphragmatic adhesions, although small inflammatory foci are still present. On the inferior surface and around the gall bladder and portal vein, Schistosoma eggs are abundant. Some lie in minute venules, others appear to lie free in the areolar tissue and are encapsulated by fibrosis. A few lie in foreign-body giant cells. Small dense scars of similar size are numerous, rarely having a deformed egg. The wall of the portal vein has a marked atherosclerotic process. The thrombus is undergoing active organization.

Pancreas: The pancreas is represented by a group of adenofibromatous masses of compressed proliferating ducts and islets. Few groups of acinar cells remain. In the peripancreatic fatty areolar tissue, there are several small nodular scars, similar to those found in the perihepatic tissues. Eggs are absent.

Diagnoses.—Massive calcinosiderotic schistosomal splenomegaly; schistosomal cirrhosis of liver; schistosomiasis of upper abdominal retroperitoneal tissues; chronic thrombosis of splenic, portal, and mesenteric veins; ruptured esophageal varix with exsanguination; varices of esophagus; ascites; chronic interstitial pancreatitis; melanosis of colon; cholemic nephrosis.

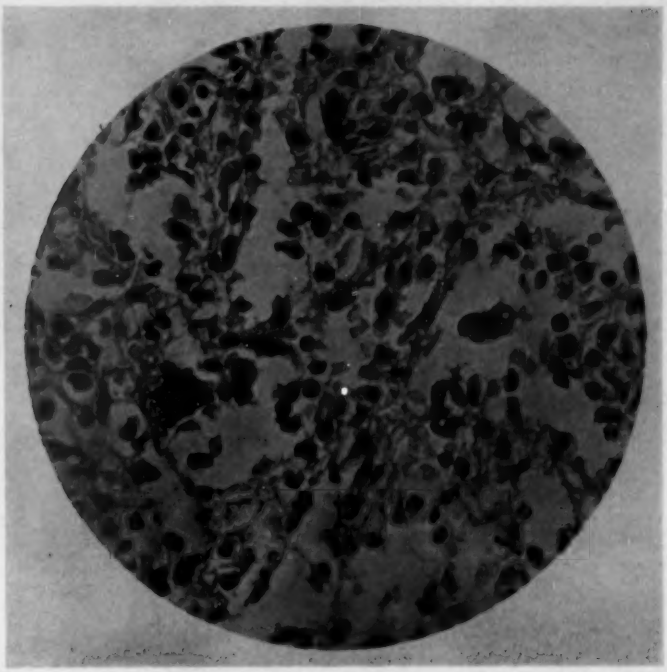

Fig. 3 (Case 1).—Phagocytized schistosomal pigment within large hyperplastic reticulum cell of pulp. × 450.

Comment.—This case is an excellent example of a condition masquerading as a clear-cut cirrhosis of the liver which proved pathologically to be primarily a massive schistosomal splenomegaly with portal hypertension, and termination by exsanguination from a ruptured esophageal varix. On resurvey from the clinical standpoint, one is impressed by the many missing diagnostic links in the chain of cirrhosis, viz., spider angiomata, axillary and body alopecia, absence of pubic hair and its female distribution, genital atrophy, gynecomastia, and hepatomegaly. In view of the apparent liver disease and the endemic prevalence of schistosomiasis in Puerto Rico, schistosomiasis was considered but never proved ante mortem. A rectal biopsy may have proved more definitive. Pathologically, the more marked extrahepatic distribution of the schistosomal infestation, especially in the retroperitoneal soft tissues of the upper abdomen, was especially noteworthy.

Case 2.—The patient, a Puerto Rican man aged 43, entered New York City Hospital on Jan. 2, 1951, with a history of sudden weakness and dizziness and vomiting of bright-red blood while walking in the street.

The past history revealed that at 19 years of age he contracted syphilis and was treated with arsenicals and bismuth for two years. He married at the age of 30. There were seven children, all free of the stigmata of congenital syphilis. At 38 years of age, an injury to the left leg was followed by persistent edema. Later, a diagnosis of filariasis was made. During his 40th and 41st years he experienced recurrent episodes of colicky, nonradiating epigastric pain followed by vomiting of sour watery material; hematemesis and melena were absent. The attacks lasted one to five days, and he did not eat during these episodes. The intervals between attacks were symptomless. He was hospitalized during the last bout. Physical examination at this time revealed supraumbilical tenderness without rigidity, absence of hepatomegaly, and Argyll Robertson pupils. The Wassermann reaction of the spinal fluid was positive. All other physical findings, laboratory tests, and roentgenologic examinations were within normal limits. He was considered to be suffering from tabes dorsalis

Fig. 4 (Case 2).—Fully developed calcinosiderotic lesion of trabeculae present in both cases. In the upper focus, the arteries are distinct and the veins obscured. In the lower focus, only thin-walled channels are easily identified. The pigments are spread diffusely but are denser at the periphery. A circumferential zone of recent hemorrhage extending into the pulp surrounds each lesion. × 20.

and tabetic crises and was given a course of penicillin therapy. He remained in good health for the next two years and gained 10 lb. in weight.

The onset of the current illness was sudden and occurred three days prior to admission to the hospital. He was seized with epigastric distress and a sense of constriction, which persisted for 72 hours and was relieved by milk and fluids. It was followed by the episode which brought him to the hospital.

Physical examination on admission revealed marked pallor, a blood pressure of 80/50, and a pulse rate of 100 a minute. The abdomen was soft and was not tender or rigid. Abdominal masses or organs were not palpable. There was no jaundice, ascites, or spider angiomata.

The laboratory examinations showed a hemoglobin of 6 gm. per 100 cc., a red cell count of 2,160,000, and a hematocrit reading of 25 vol. per cent. Thymol turbidity was 5 units, alkaline phosphatase 1.6 Bodansky units, serum albumin 4.6 gm., and serum globulin 2.2 gm., per 100 cc., and the blood Wassermann reaction was negative. Roentgenologic examination of the thorax and upper gastrointestinal tract was noncontributory.

Following transfusion, the hemoglobin rose to 8 gm. per 100 cc. and the blood pressure to 120/65. As far as could be ascertained, bleeding had stopped, although the stools continued tarry. His condition remained stabilized until the eighth day, when another massive hematemesis occurred; this again responded satisfactorily to transfusions of 1000 cc. of blood and 1500 cc. of plasma. The blood pressure returned to 110/65.

A third massive hematemesis occurred on the 11th hospital day, again responding to the transfusion of 1000 cc. of blood. The hemoglobin values, however, remained low, between 4 and 5 gm. per 100 cc. A week later an esophagogram showed the typical findings of varices in the lower third of the esophagus.

Since he showed manifest evidences of continued blood loss, a Patton tube was passed. The bleeding appeared to be controlled, and the blood pressure remained constant at 110-120 mm. Hg systolic and 70-75 mm. Hg diastolic.

The fourth massive hematemesis occurred on the 24th day of hospitalization. He again responded to transfusions of blood and plasma and was temporarily improved for two days. This was followed

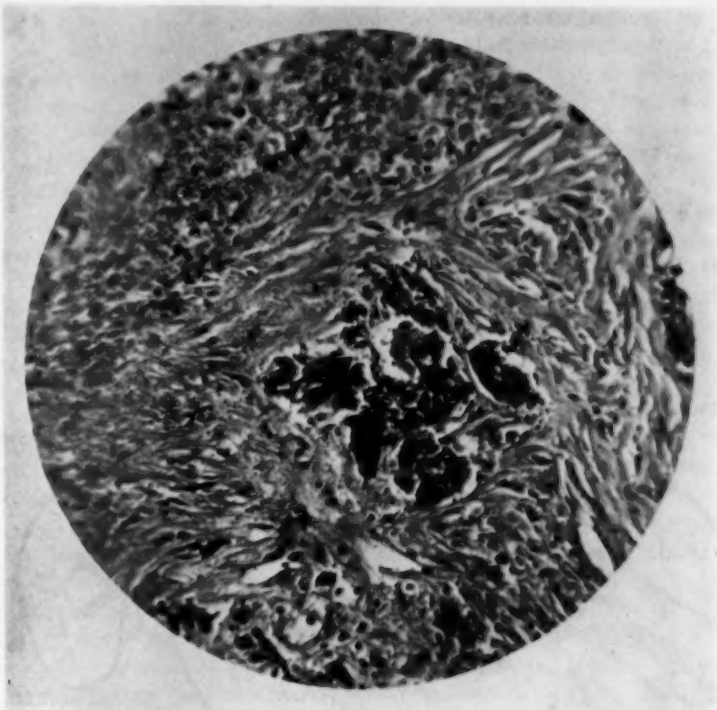

Fig. 5 (Case 2).—Foreign-body-cell granuloma. The foreign-body giant cells contain both iron and calcium. × 260.

by the fifth, and fatal, episode of uncontrollable bleeding, and death followed in five hours.

Autopsy (performed 13½ hours after death.)—Only the pertinent findings are abstracted.

The body is that of a well-developed, well-nourished white man appearing about the stated age of 43. Jaundice and ascites are absent.

The spleen is normal in shape, firm, and enlarged, weighs 500 gm., and has a slightly thickened capsule. Perisplenic adhesions are absent. On section, the cut surface is firm and red-brown in color; the trabeculae are prominent and the follicles obscure. Scattered throughout the organ are innumerable small, well-demarcated, rusty-brown foci.

The portal system is extensively thrombosed. From the hilus of the spleen to the hilus of the liver the veins are completely occluded. Thrombosis involves the cystic vein and extends along one side of the superior mesenteric vein; one small tributary is completely occluded. The short gastric veins are distended and thrombosed. The oldest part of the venous occlusion is in the splenic vein, where it is gray, firm, and tightly adherent. In the portal, cystic, and mesenteric veins, the thrombus is softer, redder, and tightly adherent. In the short gastric veins, the thrombi are only lightly adherent. The hepatic, splenic, and superior mesenteric arteries are normal.

The liver weighs 1550 gm. and appears normal. At the point where the thrombosed portal vein crosses the common duct, the lumen of the duct is narrowed. Above this point, the common and hepatic ducts are slightly dilated, the dilation extending a short distance into the liver.

The esophagus has distended varicosities, showing two points of erosion, one at the junction of the upper and middle thirds, the other at the cardioesophageal junction. The esophagus and stomach are filled with fluid blood. The rest of the gastrointestinal tract has tarry contents.

Microscopy.—Spleen: The microscopic findings duplicate in almost all respects those of Case 1 and therefore will not be given in detail. Only those features will be mentioned which are at variance. Inflammatory reaction is much scantier and the degenerative lesions of the connective and elastic

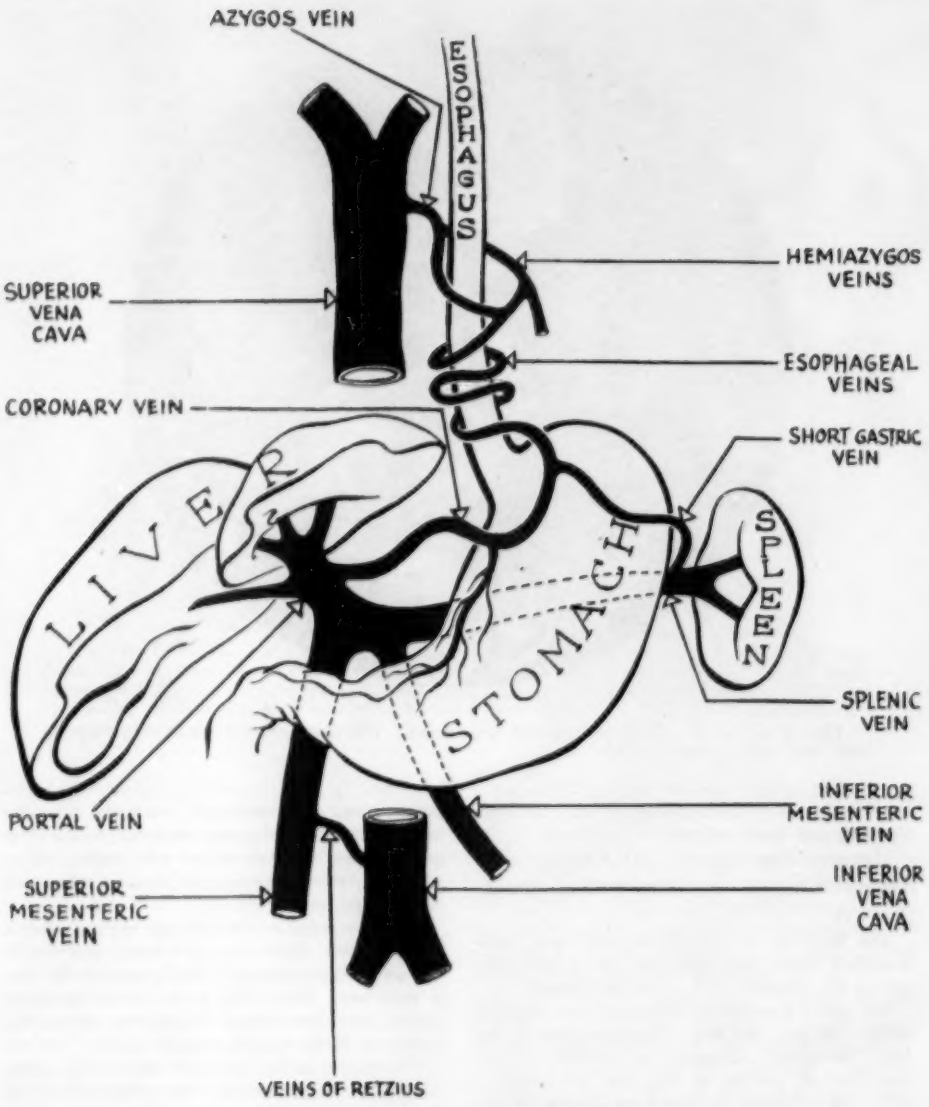

Fig. 6.—Schema of portal vein circulation.

tissues more evident. Venous channels are more difficult to identify; frequently they appear replaced by thin-walled venules. In areas of heaviest pigment deposit, foreign-body granulomata are frequent, the giant cells engulfing both hemosiderin and calcium, the calcium being more abundant. A rare artery has a calcified internal elastica. There is an absence in the pulp of phagocytic cells containing nonferrous pigment.

Portal System: The walls of the portal, splenic, and mesenteric veins have severe atherosclerosis, with many foci of calcification. The splenic vein near the hilus is surrounded by dense fibrous tissue, and the thrombus is fibrous. In other regions the

thrombus shows active organization of various ages, the youngest stage being in the mesenteric and short gastric veins. The corresponding arteries are normal.

The liver is normal.

Diagnoses. — Calcinosiderotic splenomegaly; syphilitic (?) splenogemaly; atherosclerosis of splenic, portal, and superior mesenteric veins with progressive thrombosis; ruptured varices of esophagus with exsanguinating hemorrhage.

Comment.—The presenting clinical picture was strongly suggestive of bleeding ulcer. The absence of an ulcer crater and the presence of esophageal varices as evidenced by roentgenography introduced factors compatible with hepatic cirrhosis, which was the clinical impression in this case. Death by exsanguination from a ruptured varix supported this diagnosis. As in Case 1, however, the ancillary findings were lacking. The autopsy revealed a chronic occlusive venous phenomenon affecting the spleen as the primary site and a normal liver. The etiology remained obscure. Of more than passing interest, however, was the history of syphilis and the vigorous antisyphilitic therapy administered.

Comment

The pathogenesis of massive calcinosiderotic splenomegaly has many obscure features. There are, however, certain constant findings and certain accepted associations. The lesion is trabecular in site, exhibits remote and recent hemorrhage, shows regressive changes in the fibrous and elastic tissues, with calcium deposition, and is accompanied by fibrotic changes of the pulp. It is generally accepted that the lesion is closely associated with the vascular apparatus. There is disagreement, however, as to whether the arterial or the venous element plays the major role. Symmers stressed the changes in the arterial walls and postulated that these changes impaired the circulation, leading to the escape of serum and precipitation of calcium in tissues previously ironized. It seems apparent that a relatively late phase of the process was being considered, since ironization had already occurred. Simonds found that the trabecular artery was always patent and that the first hemorrhage surrounded this vessel. He postulated that the total blood space in the spleen was greatly increased, resulting in a diminished rate of blood flow. The slowed blood streams, together with changes of the venous walls and slight local phlebitis, caused venous thrombosis, followed by hemorrhage and iron and calcium deposition. Our observations in regard to patency of the arteries are in agreement with those of Simonds. Venous thrombi were not found, although inflammatory reaction in the walls was occasionally seen. Rather, the veins tended to be obliterated. Stengel likewise noted that the small vessels tended to obliteration, although he did not specify whether they were veins or arteries. Nor does the diffuse trabecular pattern of the lesion conform to that of an infarction, the natural sequel of venous thrombosis. Sprunt demonstrated that the lesion was not confined to the vessels and that the entire trabecular tissue was involved to a much greater degree than the vessels themselves. Our observations conform with those of Sprunt. Hemosiderin was found throughout the trabeculae and was sometimes more abundant at the periphery than close to the artery and vein. All observers agree that of the pigmentary deposits, the iron precedes the calcium and that they are present in fibrous and elastic tissues which have undergone regressive changes. Ehrlich [18] stated that elastic fibers near hemorrhagic foci become impregnated with iron, which subsequently are the site of calcium deposits.

The pathogenesis of massive calcinosiderotic splenomegaly, as we conceive it, is based on the concept of a chronic venous obstructive phenomenon. The basic lesion is obstruction to the outflow of blood in the presence of an intact inflow tract. The obstruction leads to chronic engorgement, followed by its natural effects upon the fibrous and elastic elements. In the pulp,

there is a diffuse reticular hyperplasia, leading to congestive cirrhosis. The trabecular changes are slower in appearing. There is an increase of fibrous and elastic tissues, which undergo regressive changes. Because of the changed hemodynamics, an anoxic factor may play a role in the development of these regressive changes. The hemorrhages in the trabeculae are due to diapedesis or rupture of minute vessels, either venules or capillaries. Once initiated, the hemorrhagic phenomenon is progressive and extends peripherally into the peritrabecular pulp. Hemorrhage is followed by degeneration of the hemoglobin and ironization of the trabecular elements. The exudation of serum leads to the subsequent deposition of calcium on tissues already impregnated with iron.

This concept of the pathogenesis appears compatible with observations in reported cases of calcinosiderotic splenomegaly. In 8 of the 10 cases in the literature,[5,8-10] there was hepatic cirrhosis, a condition associated with portal hypertension and congestive splenomegaly. In our Case 1, the extent of hepatic cirrhosis was too limited to explain the splenomegaly. In our Case 2, hepatic cirrhosis was absent, as was true in Case 4 of Symmers[9] and the case of Fiessinger and associates.[11] In both instances, however, there was splenic vein thrombosis. Klemperer[6] stated that calcinosiderotic splenomegaly was characteristic of chronic obstruction. It is also worth noting that the splenic venous thrombosis in our cases was older than the thromboses in the other parts of the portal system. Calcinosiderotic nodules of the spleen have been found in many conditions, accompanied by minute hemorrhages, but, as noted by Brackertz,[5] they usually tend to be sparse and are not a prominent feature. The condition as seen in the massive splenomegalic form probably takes a very long period to develop, even when associated with hepatic cirrhosis. Marini's[14] case suggests this possibility. He described a case presenting all the features but one, the deposit of calcium, found in the condition under consideration.

The etiology of massive calcinosiderotic splenomegaly frequently is obscure or unknown. In most of the cases it appears as a complication of hepatic cirrhosis. Thrombosis of the splenic vein is not a necessary adjuvant. Only in Symmers' Case 3[9] were there found mural thrombi, not completely occlusive.

Infestation with Schistosoma mansoni is apparently the etiologic factor in our Case 1. The splenomegaly in this disease is usually attributed to the hepatitis.[6,15-17] Day[15] believed that the degree of splenomegaly was dependent upon the extent of involvement of the splenic vein at the hilus. It has been stated that ova in the spleen are absent[15,18] or infrequent[16,17] or occur only in massive infestation.[17,18] They have been seen in the capsule.[19] Giffen[20] claimed that ova could be demonstrated in a large percentage of cases by digestion of the spleen. In Case 1, Schistosoma eggs were not found in the spleen, but digestion of the tissues was not carried out. In the pulp there was a phagocytized nonferrous pigment, apparently similar to that described by Koppisch[17] and considered by him characteristic of this parasitic infestation. The marked predilection for the small venous radicles is a well-recognized feature of schistosomiasis. Another finding of interest worthy of mention in the etiology of calcinosiderotic splenomegaly was the unusual localization of heavy infestation in the upper abdominal retroperitoneal fatty areolar tissues. The hepatic involvement was relatively minor. The importance of retroperitoneal location as a factor in producing the splenomegaly in schistosomiasis has never been adequately investigated. Dew[21] found this region involved in a few cases, and Koppisch[17] encountered it rarely with massive infestations.

Syphilis has been implicated as the etiologic agent in some instances. In the cases reported by Symmers[9,10] all the patients were syphilitic. He believed the disease

process was an unusual manifestation of syphilitic infection. He was unable to demonstrate spirochetes. Syphilis of the spleen is very uncommon and may be gummatous or diffuse. To the best of our knowledge, calcinosiderotic splenomegaly, except for Symmers' report, has not been attributed to syphilis. Letulle [22] described an obliterative syphilitic phlebitis, not specifically of the spleen. In our Case 2 there were clinical evidence of syphilis and an obliterative lesion of the small splenic veins, unlike that of Letulle. Whether it was of syphilitic nature or not remains uncertain. Syphilis as the etiologic factor in this patient remains, however, a distinct possibility.

To evaluate the role of calcinosiderotic splenomegaly in the development of a clinical syndrome presenting many features of hepatic cirrhosis, it is apropos to review the normal physiologic hemodynamics of the portal system. The hepatic blood flow in man ranges from 1 to 2 liters per minute, with an average of 1.5 liters, or 1 cc. per gram per minute. Three-quarters of the blood reaches the liver via the portal vein; the remaining one-quarter, through the hepatic artery. There are separate currents within the portal vein, specific splanchnic areas draining into precise regions of the liver. The splenic, gastric, and inferior mesenteric veins, carrying the blood from the spleen, stomach, and left colon, drain into the left hepatic lobe. The superior mesenteric vein, carrying the blood from the small bowel and right colon, drains to the right lobe. The normal hydrostatic pressure in the portal vein lies between 80 and 120 mm. of water. The pressure in the splenic vein is slightly less. The portal system lacks valves. Consequently, the blood stream is quickly influenced by increments of pressure in the arterial and venous circuit within the liver.

Resistance to the flow of blood through the liver, whether due to cirrhosis, portal or splenic vein thrombosis, cardiac failure, or other conditions, causes a rise in portal tension to levels of 300, 400, or even as high as 600 mm. of water. The blood consequently is forced into collateral pathways, which normally are small, collapsed, and nonfunctioning. The viscera drained by this expanded venous reservoir become engorged and enlarged. One of the main abdominal organs so affected is the spleen. In the splenic segment of the portal system, the collateral channels include the short gastric veins, which anastomose with the veins of the esophagus. When distended, these veins form varices. When the hydrostatic pressure reaches 250 mm. or more of water, rupture of these varices is likely to occur.

The splenomegaly under consideration raises the question of Banti's disease. According to Banti,[23] there is a primary splenomegaly of toxic origin, which is accompanied by secondary anemia and leukopenia, terminates with hepatic cirrhosis, and has a specific splenic pathology. As defined by Osler,[24] this disease is characterized by great chronicity, progressive splenomegaly, secondary anemia, leukopenia, marked tendency to hemorrhage from ruptured esophageal varices, and a terminal hepatic cirrhosis. This concept is not at present widely accepted in American medical circles. If Thompson's [25] concept is followed, then Banti's disease is not an entity and the splenomegaly is congestive in nature and occurs as a mechanical result of hypertension in the portal and splenic veins. The commonest cause of congestive splenomegaly is hepatic cirrhosis. However, extrahepatic mechanisms can produce a portal hypertension, and ultimately a congestive splenomegaly results. Among these may be listed portal and splenic vein thrombosis, strictures and stenoses from trauma or perivenous pressure, and congenital anomalies of the portal system. The anemia would be called today a manifestation of hypersplenism. Instances have been reported, however, without portal hypertension which cannot be explained on the basis of mechanical congestion.[26,27] In still other instances there have occurred splenoportal obstruction without splenomegaly.[28] The pathologic changes

in the spleen reported by Banti are now recognized as nonspecific,[25,4] even among the European investigators,[26] who still accept Banti's original concept. Gelin [26] differentiated Banti's disease from Banti's syndrome by the presence or absence of portal hypertension. By employing the percutaneous transplenic method of portal venography, he found that many of the cases of splenomegaly he encountered had normal portal systems. These cases he termed "true" Banti's disease. The etiology was variable, sometimes a single factor, frequently several factors acting in unison. He advanced the hypothesis that repeated infections caused an increased production of antibodies in the spleen, leading to reticular hyperplasia. These antibodies reached the liver through the splenic vein and acted upon its reticuloendothelial system, producing the hepatic cirrhosis. Banti's syndrome he attributed to portal hypertension. The findings in our cases are explainable on the basis of a congestive lesion, both intra- and extrasplenic in origin. The mild or absent hepatic cirrhosis is evidence that the lesion can originate in the splenic part of the portal system and be followed by its local sequelae without necessarily occurring secondary to a hepatic cirrhosis. The most serious sequel is the development of esophageal varices.

The chief clinical importance of congestive splenomegaly is the menace of intractable hemorrhage from esophageal varices. They are observed in 80%-90% of patients with portal hypertension, are demonstrable by barium swallow in 50%, and are the cause of fatal hemorrhage in 25%-30%. Atkinson and his colleagues [27] found that transplenic venography demonstrated varices more frequently than the esophagogram or esophagoscopy, either alone or in combination. Our two cases illustrate the importance of esophageal varices, particularly in primary congestive splenomegaly. The question of differential diagnosis arises in patients presenting features of hepatic cirrhosis. If confirmatory signs, such as angiomata, changes in distribution of body hair, or estrogenic hormonal effects on breasts and genitalia, are lacking in a patient showing many signs and symptoms of hepatic cirrhosis, and having a massive splenomegaly and negative liver function tests, the possibility of the splenomegaly being primary, and not secondary, should be given serious consideration. Since the disease process may still be confined to the splenic area of the portal system, the feasibility of operative procedures should be thoroughly explored. Splenic extirpation could prove a life-saving measure.

Summary and Conclusions

Two cases of massive calcinosiderotic splenomegaly are reported and the literature is reviewed.

A concept of the pathogenesis is presented.

The etiology is frequently obscure. The splenomegaly can be caused by infestation with Schistosoma mansoni and may possibly be syphilitic in origin.

The splenomegaly may be a primary disease unassociated with other causes of portal hypertension.

Careful evaluation of symptoms suggestive of hepatic cirrhosis with outstanding splenomegaly should be carried out to determine the presence of a primary splenomegaly.

Surgical intervention in primary splenomegaly appears justified to avoid fatal hemorrhage from esophageal varices.

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Transmission of Rabies from Artificially Infected Bats to Syrian Hamsters

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Studies of rabies outbreaks in cattle and human beings in Central and South America have shown that vampire and fruit-eating bats may be the vectors of the virus.[3-6] Reagan et al.[9,10] demonstrated the ability of nonsanguivorous bats to become infected experimentally. Brueckner et al.[1] showed that the cave bat (Myotis lucifugus), experimentally infected, could harbor virus in the salivary gland. This work was later confirmed by Stamm and his co-workers,[13] and these investigators also demonstrated rabies in the saliva. Scatterday,[11] and Scatterday and Galton,[12] and Burns et al.[2] recently described recovery of rabies virus from insectivorous bats in Florida and Texas. The Florida studies were made after a positive diagnosis was obtained in a bat which had bitten a boy in July, 1953. This specimen proved to be a Florida yellow bat (Dasypterus floridanus). A survey of the indigenous bat population conducted over an extended area of the ranch where the biting occurred netted 384 bats. A positive rabies diagnosis was obtained in six specimens, five of which were the Florida yellow bat and one of which was the Seminole bat (Lasuirus seminolus). Kough [6] obtained a positive diagnosis of rabies from a hoary bat (Lasuirus cinereus, Beauvois) which had bitten a person in Pennsylvania during 1953. Sulkin and Greve [15] have reported the occurrence of a case of rabies transmitted to a human being by the bite of a bat.

In spite of the fact that the rabies virus has been isolated from the brain, salivary glands, and saliva of experimentally and naturally infected bats, and that they are known to attack human beings, there is not sufficient evidence to prove that rabies can be spread by the bite of the bat. There has been only one report by Sulkin and Greve, as cited above, in which rabies was transmitted to a human being by a naturally infected bat.

The following experiment was undertaken to determine whether bats infected with rabies could transmit the virus of rabies to a susceptible mammalian host by biting. Suckling hamsters were used as the host because they are easily handled and are highly susceptible.

Materials and Methods

The rabies virus in this study was Strain V-308 isolated from a dog brain in routine diagnostic work at the Live Stock Sanitary Service Laboratory. Prior to this study, it had been passed five times in Swiss albino mice. Ten 3-week-old Swiss albino mice were inoculated intracerebrally with 0.03 ml. of the fifth mouse passage. After six to seven days symptoms of rabies appeared and the mice were killed. The brains were removed aseptically, stained with Sellers' stain,[14] examined under an optical microscope, and found to contain numerous Negri bodies. A 20% suspension was made with isotonic saline from the remainder of the mouse brains. The suspensions were centrifuged at 2000 rpm for five minutes in an angle centrifuge. The supernate was used for the present study, the titer of which was $10^{-2.5}$ when inoculated intracerebrally into Swiss albino mice less than 3 weeks of age.

Twelve healthy cave bats (Myotis lucifugus) were stored in the refrigerator for several days before inoculation. They became fully active within 20 minutes after removal from the refrigerator and were placed in individual jars in preparation for inoculation with rabies virus. Bats 1

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From the Virus Laboratory, Live Stock Sanitary Service, University of Maryland.

TABLE 1.—*Reaction of Artificially Infected Nonsanguivorous Bats (Myotis Lucifugus) to Rabies Virus (V-308 Strain)*

Bat No.	Inoculum Subcutaneous (Cervical Region), Ml.	Rabies Symptoms	Microscopic Examination (Negri Bodies)
1	0.1	+, 10 days	+
2	0.1	+, 10 days	+
3	0.1	+, 10 days	+
4	0.1	+, 10 days	+
5	0.1	+, 14 days	—
6	0.1	+, 14 days	+
7	0.1	+, 14 days	+
8	0.1	+, 14 days	+
9	0.1	+, 14 days	+
10	0.1	+, 14 days	+
	Normal brain		
11	0.1	—	—
12	0.1	—	—

to 10 inclusive were inoculated subcutaneously in the cervical region with 0.1 ml. of the 20% suspension of rabies virus. Bats 11 and 12 were inoculated with normal mouse brain suspension. The bats were housed in glass jars with wire-mesh lids. Their food consisted of a homogeneous mixture of bananas, Pablum, cottage cheese, and evaporated milk. They were supplied with plenty of water, and paper toweling was suspended in the jar for clinging. All of the inoculated bats showed tremors, and some exhibited paralysis between 10 and 14 days after inoculation. Control bats 11 and 12 remained normal throughout the experiment (Table 1). On the day of appearance of rabies symptoms the bat was allowed to bite suckling hamsters, which were then placed with the parent. Saliva was harvested from the bat by swabbing the oral region with isotonic saline to which appropriate amounts of streptomycin and penicillin had been added to kill bacteria. The bat was killed, and the brain and salivary glands were removed. The oral saline washings from the bat were injected intracerebrally into six Swiss albino mice. The brain and the salivary glands were kept separate and were triturated with Alundum (fused aluminum oxide) in a mortar, diluted with isotonic saline, and injected into Swiss albino mice by the intracerebral route (Table 2). The same procedure was carried out for the remaining infected bats (2 to 10 inclusive) upon appearance of rabid symptoms, as well as with control bats (11 and 12). The 12 groups of suckling hamsters with parents were checked twice daily. Groups 3, 6, 8, 9, and 10 were discarded because the parent had eaten all of the sucklings on the second or third day after the bat's biting. Groups 1, 2, 3, 4, 5, 7, 11, and 12 were checked daily for symptoms of rabies. The sucklings, subjected to the bat bites at the age of 4 days, were weaned at 16 days of age. Up to the 16th day, 13 sucklings had manifested rabid symptoms and were killed. Negri bodies were found in "touch" smears of brains. Each brain was triturated and suspended in isotonic saline for inoculation of 3-week-old Swiss albino mice (Table 3). The remainder of the sucklings appeared normal after a 21-day observation period. Brains of these and of the controls were removed, pooled, and injected intracerebrally into 3-week-old mice. The mice appeared normal after a 14-day observation period and were discarded.

Results

All cave bats exposed to a street virus strain of rabies showed rabid symptoms between 10 and 14 days. Examination of their brains revealed Negri bodies in bats 1, 2, 3, 4, 6, 7, 8, 9, and 10. No Negri bodies were found in bat 5 or in the controls. Saliva from infected bats 1, 2, 3, 4, 5, and 10 produced rabies in mice after intracerebral exposure to the saline washings. Saliva from bats 6, 7, 8, and 9, upon intracerebral injection into mice, produced no evidence

TABLE 2.—*Confirmation of Presence of Rabies Virus (V-308 Strain) in Artificially Infected Nonsanguivorous Bats (Myotis Lucifugus)*

Bat No.	Salivary Glands* Mouse No. Pos.	Salivary Glands* Negri Bodies (Mouse Brain)	Saliva Mouse No. Pos.	Saliva Negri Bodies (Mouse Brain)	Brain† Mouse No. Pos.	Brain† Negri Bodies (Mouse Brain)
1	6/6	+	6/6	+	6/6	+
2	6/6	+	6/6	+	6/6	+
3	5/6	+	6/6	+	3/6	+
4	4/6	+	6/6	+	6/6	+
5	3/6	+	5/6	+	6/6	+
6	4/6	+	—	—	6/6	+
7	6/6	+	—	—	4/6	+
8	6/6	+	—	—	6/6	+
9	2/6	+	—	—	6/6	+
10	5/6	+	5/6	+	6/6	+
11	—	—	—	—	—	—
12	—	—	—	—	—	—

*0.03 ml. inoculum.
†0.03 ml. inoculum of 20% suspension in isotonic saline. Rabies symptoms appeared in the mice between 6 and 14 days. All mice showing no rabies symptoms were discarded after a 21-day observation period.

TABLE 3.—*Reaction of Suckling Syrian Hamsters to Rabies Virus (V-308 Strain) from Nonsanguivorous Bats (Myotis Lucifugus) Transmitted by Biting*

Bat No.	Appearance of Rabies Symptoms in Suckling Hamsters After Biting			
	No. of Sucklings	Negri Bodies	Mouse Inoculation	Negri Bodies (Mouse Brain)
1	4/5	+	5/5	+
2	2/3	+	4/6	+
3	0/7	(eaten by parent)		
4	4/7	+	2/5	+
5	5/5	+	5/5	+
6	0/6	(eaten by parent)		
7	1/6	+	1/6	+
8	0/6	(eaten by parent)		
9	0/6	(eaten by parent)		
10	0/6	(eaten by parent)		
11	0/8	—	—	
12	0/8	—	—	

of rabies after a 21-day observation period. Salivary glands and brains from the bats also produced rabies in Swiss albino mice by intracerebral inoculation. All control bats were negative throughout the experiment. Suckling hamsters bitten by the bats showed rabies symptoms between 10 and 14 days. Examination of these suckling hamster brains prior to inoculation of Swiss albino mice revealed numerous Negri bodies. Mice injected with these brain suspensions showed rabies symptoms (Tables).

Summary

From this experiment, it is apparent that cave bats experimentally infected with a street virus strain of rabies transmitted rabies to suckling hamsters by biting. Fifty per cent of the 26 suckling hamsters bitten by rabid bats developed rabies. All controls were negative. Therefore, it appears that rabies can be transmitted by the bite of the infected nonsanguivorous bat. Since the high percentage of sucklings showed symptoms within a few days, it seems obvious that rabies in all of these sucklings developed from the bite of the bat, and not from the bite of another infected suckling.

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Infantile Endocardial Fibroelastosis

A Suggested Etiology

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During the past several decades idiopathic cardiac hypertrophy of infancy with and without endocardial sclerosis has been the subject of a constant stream of reports, which during the last 10 years has increased in volume, for the most part, in American journals. The term idiopathic cardiac hypertrophy with endocardial sclerosis has been virtually replaced by "endocardial fibroelastosis." This change in terminology is more than a simple shift in emphasis from one to another anatomic characteristic. It is the reflection of a different concept of etiology and pathogenesis.

Prior to popularization of the term "endocardial fibroelastosis" the cardiomegaly was considered idiopathic and primary, the endocardial lesion secondary. The newer viewpoints are well represented in the reviews of Craig,[1] Gowing,[2] Johnson,[3,4] Kempton,[5] Edmunds and Seelye,[6] Thomas et al.,[7] and Rosahn.[8] Most are agreed that, for these cases, the diagnosis "idiopathic cardiac hypertrophy" is no longer useful, and that many so designated are in reality instances of endocardial fibroelastosis, the result of "a primary abnormality of foetal development, an endocardial dysplasia of unknown etiology."[2] It is further postulated that "the presence of diffuse endocardial fibroelastosis almost invariably causes cardiac hypertrophy in the adjacent ventricular musculature, probably due to the increased work necessary to contract and expand the thick fibroelastic tissue of the endocardium."[4]

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Another speculation[9] suggests that fibroelastosis closes the ventricular openings of the arterioluminal vessels and Thebesian veins, interfering with the myocardial blood supply, thus leading to injury of the myocardium and resulting in hypertrophy.

Johnson,[4] one of the leading proponents of an anoxic etiology, summarized his concept: "Endocardial fibroelastosis always develops in hearts having associated malformations which could produce endocardial anoxia."

With the advent of this emphasis upon fibroelastosis, it has become customary to lump together all instances of this endocardial lesion, regardless of the presence or absence of other lesions (for example, aortic coarctation[10] and aortic or mitral stenosis[11]). The inevitable result of this contemporary elevation of "endocardial fibroelastosis" to the status of a nosological entity has been the suggestion that it represents a form of "collagen disease."[12,13] Interestingly, Hill and Reilly[12] reached their conclusion from a study of an infant, while Becker et al.[13] were concerned with adults.

The extent of disagreement characterizing the problem is well illustrated by Johnson's[4] conclusion that there is no evidence that endocardial fibroelastosis is a true congenital anomaly and Rosahn's[8] statement that the evidence strongly suggests a genetic determinant.

Endocardial fibroelastosis occurs in adults,[7,13-15] and many have recognized morphologic similarities to the infantile lesion. Most agree with Gowing,[2] however, that in the adult it probably has a different, but likewise unknown, etiology.

With the exception of Johnson[3] and Gross,[16] almost all American and English contributors have ignored the German language literature. Indeed, it was only after the etiologic concepts to be presented in this paper had been conceived that I explored this source of information. Ribbert,[17] in 1924, reviewed the older literature, writing:

> Königer had already conceived of the diffuse endocardial sclerosis as a form of vicarious replacement of a weakened myocardium. Recent investigations have uncovered support for this view. . . . That dilatation of the cardiac chambers associated with hypertension may be related to the lesion is apparent, since endocardial sclerosis is frequently far advanced in the left atrium of severe mitral stenosis.

Hubschmann[18] in 1917 had noted that endocardial thickening, particularly of the left ventricle, was not uncommonly seen in the dilated hearts of diphtheria. He believed this lesion might best be considered a reaction to dilatation of the chamber. Böger[19] observed that dilatation of the cardiac chamber was the cause of endocardial fibroelastosis, and that the role of hypertension, if any, was indirect by way of causing cardiac failure and dilatation. According to Böger, Nagryo drew a comparison between the blood vessels and the heart, referring to the fibroelastosis of the heart as a "functional diffuse elastic endocardial sclerosis."

Since virtually the only support for this hypothesis is teleologic and/or coincidental, it is not surprising that at least in the English-speaking countries the hypothesis has been completely superseded by the current "congenital genetic and/or anoxic" concepts.

Material

Because of evidence which will be discussed in the body of the paper, it was concluded that endocardial fibroelastosis could best be explained on a mechanical basis. To investigate this possibility, the following material was obtained and studied. Between Jan. 1, 1949, and Dec. 31, 1954, 12 unequivocal examples of idiopathic cardiomegaly with endocardial fibroelastosis were recorded in the protocols of the departments of pathology of the Cincinnati General Hospital and the Cincinnati Children's Hospital (Table 1). In addition, the enlarged hearts of two siblings examined at autopsy in the department of pathology of the Duke University School of Medicine were studied, one with and the other without endocardial fibroelastosis (Table 1). Two other cases encountered during the writing of this paper were included in the study: N55-383, from the Cincinnati General Hospital, and S56-55, from the Office of the Coroner of Cincinnati (Table 1). In addition to these examples of endocardial fibroelastosis, two other kinds of hearts with endocardial fibroelastosis were examined. Six were examples of ectopic left coronary artery arising from the pulmonary artery (Table 6), and six were hearts with aortic atresia or severe stenosis with hypoplastic proximal aorta, mitral stenosis, and small left ventricle with endocardial fibroelastosis (Table 7). Two instances of miniature right ventricle with endocardial fibroelastosis were likewise studied (Table 7).

TABLE 1.—*Fifteen Cases of Idiopathic Cardiomegaly with Endocardial Fibroelastosis*

Case	Age	Sex	Body Wt., Gm.	Heart Wt., Gm.	Theoretical Heart Wt. (Gm.) by		Index of Cardiomegaly	
					Age	Body Mass	Heart Wt. / Ht. Wt. Age	Heart Wt. / Ht. Wt. Body Mass
N50-104	5 mo.	F	4700	105	29	22	3.6	4.8
N52-260	7 mo.	F	6600	90	34	30	2.6	3.0
N52-494	5 wk.	M	4575	70	20	20	3.5	3.5
N54-122	11 mo.	F	6491	135	40	29	3.37	4.6
C49-35	12 mo.	F	8228	90	44	37	2.0	2.4
C49-58	12 mo.	F	7200	110	44	30	2.5	3.6
C49-79	7 mo.	M	7700	110	34	31	2.9	3.5
C50-58	3 mo.	F	4427	70	23	20	3.0	3.5
C50-93	7 mo.	F	6260	160	34	27	4.7	5.9
C52-43	4 ½ mo.	M	5217	64	26	23	2.28	2.8
C52-90	4 mo.	M	6046	125	27	29	4.6	4.3
C53-93	2 ½ mo.	M	3636	53	23	19	2.3	2.8
N55-383	12 mo.	F	8310	100	44	35	2.3	2.8
S56-55	3 mo.	M	4680	116	23	23	5.0	5.0
				Duke Hospital				
5180*	4 mo.	M	4555	73	27	20	2.7	3.6
5730	7 ½ mo.	M	6060	182	35.5	24.5	3.5	6.2

* Without endocardial fibroelastosis.

In all, 29 instances of infantile or neonatal hearts with endocardial fibroelastosis were reviewed.

Idiopathic Cardiomegaly with Endocardial Fibroelastosis

Characteristic, at the autopsy table, of the 16 cases (Table 1) is a hugely enlarged heart, which in the severest cases (Fig. 1) compresses almost all of the left lung and encroaches markedly upon the right. Immediately apparent (Fig. 2) is striking dilatation of the left ventricle, with its tendinous-appearing endocardium thickest near the base of the interventricular septum. Dilatation and endocardial sclerosis are usually present in the left atrium. The trabeculae carneae and the papillary muscles of the dilated ventricle are flattened and partly obliterated. The fusion of the papillary muscles to the mural endocardium of the dilated chamber is so marked that it has

Plate I

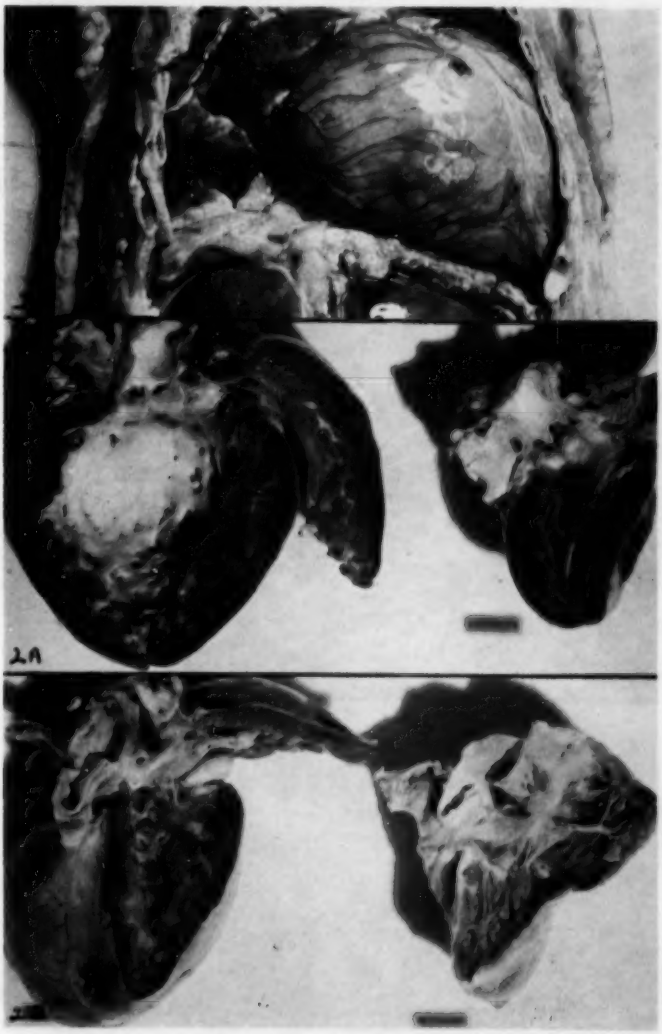


Fig. 1 (Case 5730).—The unusual degree of cardiomegaly, relative to the patient's size, is illustrated by the 152 gm. heart in a 7-month-old infant, weighing 6050 gm. The left lung is compressed and hidden by the heart, which encroaches upon the right lung and flattens the diaphragm.

Fig. 2.—*A*, on the right is the normal left ventricle of a 7-month-old infant. The endocardium is transparent. On the left is the left ventricle of the heart in Figure 1. The marked dilatation and endocardial fibroelastosis is evident.

B, the right ventricles of the hearts of *A*. The right ventricular endocardium of the enlarged heart is normally transparent and the right ventricle only slightly dilated.

been interpreted [1] as an anomalous development. The rounding of the left ventricular apex lends a globular configuration to the heart, which, internally, is further amplified by the flattening of the formerly corrugated surface.

The valves are velamentous and obviously not involved by the sclerosing endocardiosis. A relative insufficiency of the mitral valve may, of course, be present. In many instances, reported in the literature, the valves may participate in the sclerosis, resulting in stenosis and/or insufficiency. Such cases complicate the problem of pathogenesis, since the valve change may be primary; such examples therefore have been omitted from the study.

The right atrium and ventricle usually present no lesions or abnormalities. Occasionally, in the largest hearts, some thicken-

Plate II

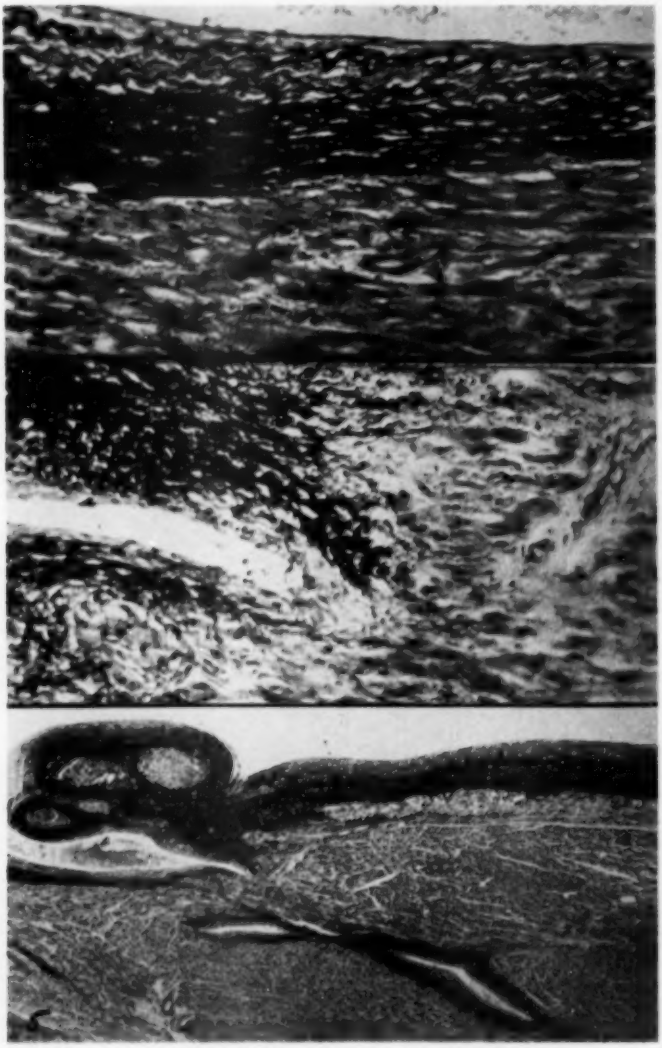


Fig. 3 (Case N55-383; Table 1).—The elastosis and thickening of the left ventricular endocardium and preservation of its myocardium are illustrated.

Fig. 4 (Case C52-90; Table 1).—The characteristic disappearance of the endocardial elastosis at the acute angle of juncture of a trabeculum and the compact myocardium is interpreted as an expression of low tension at this point.

Fig. 5 (Case C52-90).—The trend toward obliteration of the internal corrugations with smoothing of the internal surface of the heart is demonstrated by the fusion of trabeculae carneae.

ing of the right ventricular endocardium may be seen (C50-93); this, however, is rarely even remotely comparable to that of the left ventricular endocardium.

Histologically the myocardium is essentially intact. The border between the left ventricular muscle and the endocardium is sharply defined. The connective tissue septa may increase in width.

The endocardium (Fig. 3) is markedly thickened, containing many elastic fibers, which lie parallel to the endothelium. The thickness and number of the individual fibers diminish toward the lumen. Characteristic is the absence or diminution of the elastica at the angle of clefts formed by the trabeculae carneae and the compact mural myocardium (Fig. 4). Just as characteristic is the encircling of the cross-sectioned trabeculae by the fibroelastic endocardium (Fig. 5); the persisting muscle fibers are vacuolated and sometimes difficult to recognize (Fig. 6). The same changes are seen in the wall of the left atrium.

The size attained by these baby hearts has never been properly evaluated. Perusal of the literature leaves one with the impression that all realize the hearts are very large, but nowhere is it noted that, correlated with the patient's mass, they are among the largest hearts recorded. For example, the 175 gm. heart of a 7-month-old infant,[20] assuming a 50 percentile weight for that age and sex of 7.7 kg.,[21] would in an adult of 160 lb. (72.6 kg.) weigh about 1765 grams!* Case C50-93 under the same conditions, reckoning however from its actual weight, would have a heart of 1973 gm.!* In contrast, a relatively small heart, as this series goes, C53-93, at 2½ months, 53 gm., would in a 160 lb. adult weigh 1444 gm!*

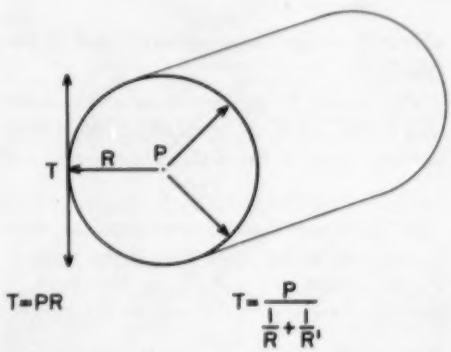
Hearts of these magnitudes are not concentrically enlarged but, as Figure 2 clearly shows, are very much dilated. The unusual degree of dilatation is attested in almost every pertinent publication.

The meaning of marked cardiac dilatation for the pathogenesis of endocardial fibroelastosis can best be understood if the significance of the internal structure of the ventricles is appreciated and some simple principles of hydraulics are applied.

In 1892, Woods,[22] in a neglected paper, explored the functional implications of cardiac structure. More recently (1952), and quite independently, Burch, Ray, and Cronvich[23] described a similar, but more extensive, investigation. It is interesting to note that, while Woods applied Laplace's law of hydrostatics to the heart for the first time in 1892, it was neglected for 60 years, when Burton[24] applied it to the muscular arteries. In 1954 Willis[25] utilized it to explain the localization of arteriosclerotic plaques at arterial bifurcations.

In its fundamental form[24] Laplace's law is (*a*) $T=PR$, where T is the tension per unit length engendered in the wall of a cylinder, the tension being measured at right angles to the hydrostatic pressure, P. P acts perpendicularly to the cylinder wall, whose radius is R (Text-Figure 1).

The importance of radius (R) is illustrated by the structure of a hydraulic braking system. The master cylinder wherein



Text-Fig. 1.—A simplified vector diagram illustrating the components which enter into the equation $T=PR$. P is the hydrostatic pressure acting at right angles to any given locus on the wall of the cylinder over a distance R (radius of cross section) and exerting a tension, T, which tends to disrupt the wall of the cylinder.

* This and the following weights are arrived at by a simple proportion. The adult weight of 160 lb. was arbitrarily chosen. Because they are abnormal hearts, the normal infantile and adult heart/body mass ratios were ignored.

pressures up to 500 lb/sq. in. are generated has a radius of ½ in. To contain this pressure the cylinder wall is ¼ in. thick and is made of steel. The transmission tubes carrying the same pressure may be made of flexible copper or aluminum with a radius of 3/32 in. and a wall thickness of only 1/16 in. If the transmission tubes were of the same radius as the master cylinder, they would rupture when subjected to pressures far below those safely carried in small caliber tubes.

For simplicity's sake, the heart chambers may be likened to spheroids wherein each locus of their internal surfaces has two radii, a small one, R, and a long one, R^1; then *(a)* becomes

$$T=\frac{P}{\frac{1}{R}+\frac{1}{R^1}} \qquad (b)$$

It is obvious from *(b)* if the pressure within the left ventricle remains constant or nearly so, as it must if systemic blood pressure is to be maintained, the tension engendered in the wall of the heart must rise as the diastolic volume increases; hence increased radii.

The magnitude of this rise may be derived in the following manner †: Assuming the heart chambers to be spheres, then the force exerted by the blood against the wall is

$$F=P\pi R^2 \qquad (a)$$

where P is the blood pressure and R the radius.

The stress, T, per unit area of the heart wall is the force, F, divided by the cross-sectional area of the wall, A, or

$$T=\frac{F}{A}=\frac{P\pi R^2}{A} \qquad (b)$$

If the myocardium is considered an elastic material in the sense that if the volume, V, of muscle is constant, its thickness, t, varies inversely with the surface area, then

$$V=4\pi R^2 t \qquad (c)$$

since

$$A=2\pi Rt$$

then

$$A=\frac{V}{2R} \qquad (d)$$

Substituting d into b,

$$Tt=2\pi R^2\frac{P}{V} \qquad (e)$$

or

$$Tt\propto R^2 \qquad (f)$$

Applying *(f)*, it appears that in a ventricle whose radius has doubled, with blood pressure maintained, the mural tension must increase not two times but eight times!

Because of the above conclusions, it would appear that any mechanism operating to increase the effectiveness of the myocardium or decrease the residual, and therefore diastolic, blood volume would obviously be advantageous to cardiac function. The normal heart is provided with such mechanisms by virtue of its internal structure.

Woods,[22] in his remarkable dissertation, concisely described these mechanisms in the following quotations, referring to the columnae carneae:

> These muscles, which stretch across the cavity of the heart from wall to wall, exert their influence more immediately in the blood by pulling more directly on the ventricular wall, and consequently are more efficient than if they lay on the outside of the wall itself. . . .
>
> It may be objected that placing muscles inside the heart cavity would necessitate its enlargement for the purpose of making room for them. But a little consideration will show this is not the case. For suppose the heart a sac with smooth walls, it would be quite impossible for it to contract so as to obliterate or even almost obliterate its cavity when expelling blood under considerable pressure, owing to the difficulty of approximating the origins and insertions of the fibres, especially of the innermost layers, so that the only difference their presence makes consists in diminishing the quantity of residual blood in the heart.

Referring to the musculi papillares, Woods wrote:

> The musculi papillares are usually considered as having only to do with controlling the mitral and tricuspid valves. But they must also aid in expelling the blood. For the exertion necessary to prevent the valves from flapping back into the auricles must also react on the ventricular wall

† Mr. Calvin C. Bopp, E. E., manager of the department of research and development of the Crosley Division of Avco, Cincinnati, adapted the method from Woods' analysis.[22]

and help it in its effort at contraction. They must then be looked upon as having the double function of controllers of the valves and true working muscles of the contracting heart itself.

Burch et al.[23] inquired into the functional significance of the same anatomic structures and arrived at the same conclusions. The studies of Berry[26] and Patten et al.[27] demonstrate a somewhat similar action in respect to residual blood volume by the cardiac jelly in the valveless tubular heart of the 50-hour chick embryo.

The anatomic features of the internal surface of the heart are accentuated in hypertension or valvular disease without failure; however, when the load becomes too great for the hypertrophic myocardium, dilatation occurs. At this point the effectiveness of these extremely important aids to normal function is reduced. As the heart grows more globular and the trabeculae carneae subtend a diminishing arc of mural myocardium and the papillary muscles are pressed against the wall and gradually effaced, the heart fails.

The magnitude of the increased work added to a dilated heart is readily grasped from a table calculated by Burch, Ray, and Cronvich.[23] The authors emphasize that the figures represent approximations. The relationships of the values in this Table are valid for infantile hearts.

TABLE 2.—*Energy per day Necessary to Overcome Left Ventricular "Load" in the Normal and Dilated Heart**
Cardiac Rate 60/Min.

Work/Day	Normal Heart Systolic B.P. 120 mm. Hg	Dilated Heart Diastolic Volume 500 cc. Systolic B.P. 120	Dilated Heart Diastolic Volume 500 cc. Systolic B.P. 220
Ergs	43.2×10^{10}	388.8×10^{10}	691.2×10^{10}
Kg.-Cal.	10.37	93.6	166.4
Ft.-lb.	32,000	288,000	512,000

* From Burch, Ray, and Cronvich.[23]

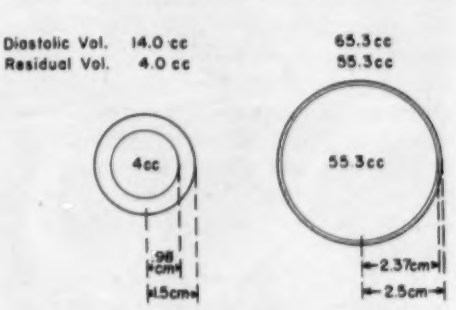
Contributing in good measure to the loss of efficiency of the heart, since it does no more effective work at a much higher level of energy consumption, is the fact that with dilatation the force required to attain peak systolic pressure is greater than that needed to achieve diastolic pressure. In the normal heart the reverse is true. Burch et al.[23] demonstrated this as follows:

$$F=P\times A$$

where F is the force exerted by the ventricular muscle against the blood at P, hydrostatic pressure, covering the entire area, A, of the ventricular sphere. Since the area =

$$4\pi R^2$$
$$A\propto R^2$$

As the heart contracts from the end of its isometric phase, A rapidly diminishes in size $A\propto 1/R^2$. Thus, despite the progressive rise of P as it approaches the systolic peak pressure, the reduction of A is so much greater that when it is reached, F is actually smaller than at the beginning of systole. This advantage of the cardiac pump over mechanical pumps is likewise lost with significant dilatation of the heart.[23] Such



Text-Fig. 2.—A schematic diagram illustrating the fact that with increased diastolic volume the change in the cardiac silhouette accompanying systole decreases, although stroke volume remains constant. It is apparent that, as the heart chamber dilates, the flexibility of its endocardium grows less important.

loss is readily demonstrated by the following examples (Text-Fig. 2): The total diastolic volume of the left ventricle of an infant heart containing 14 cc. of blood may, with each stroke, expel 10 cc., leaving a residuum of 4 cc. If in the same infant severe dilatation was present and the diastolic blood volume was 65.3 cc., then at the end of systole the residuum would be 55.3 cc. It is apparent that A in the dilated heart

has changed very little, and consequently the force necessary to achieve systolic pressure may be considerably greater than that required to equal diastolic pressure. In addition, the considerably enlarged heart must go through a relatively small excursion to expel only 15.5% of its diastolic blood volume, as against the very noticeable but normal excursion required to expel 72% of its diastolic blood. This well-known relative immobility of the markedly dilated heart accounts for the difficulties in differentiating, fluoroscopically, cardiac dilatation and pericardial effusion.[15]

The relationship [28]

$$\frac{O_2}{\text{Diastolic Blood Volume}} = \text{constant}$$

summarizes the individual aspects of impaired function brought on by dilatation, as itemized above. The formulation expresses the fact that within wide limits the myocardial oxygen consumption maintains a constant relationship to the diastolic blood volume. As the heart dilates, the myocardium must use an ever-increasing amount of oxygen. Since the effective work done is not more, and terminally perhaps less, than that done by the normal heart, myocardial efficiency falls in direct relationship to dilatation.

Is it possible, with the above anatomical-functional relationships in mind, to construct a sequence of events leading to endocardial fibroelastosis without doing violence to the laws governing the heart?

A reasonable hypothesis may begin with the postulate that the basic fault in the hearts listed in Table 1 must be sought in the myocardium.‡ Certainly it cannot in any way be associated with hypoxemia. The essential intactness of the myocardium seen in all cases of this series, as well as in almost every other report, would seem to be adequate reason to deny the significance of hypoxemia, in the sense of inadequate blood volume, as far as any effect upon myocardium is concerned. Furthermore, the endocardium is in intimate contact with the oxygenated blood of the left side of the heart, and the coronary circulation is demonstrably intact. There appears, literally, no good reason to implicate hypoxemia other than the inductive reasoning of Weintraub and Himelfarb,[9] which has been propagated by others.

If, for the moment, the thesis of a weak or inefficient myocardium is accepted, a series of events follows which reasonably may end in endocardial fibroelastosis. After birth the shift of systemic pressure, previously shared by both ventricles, entirely to the left ventricle results in its dilatation. This, in turn, leads to an increase in the load laid upon the myocardium, which is inadequate to its burden, resulting in further dilatation with another increment to its load. This is not an explosive process but one partially compensated by elongation of the myocardial fibers, with consequent increased power and eventual hypertrophy, and thus further reinforcement of the myocardial stroke. However, despite these two factors, elongation and hypertrophy of the fibers, the myocardium may still not be entirely equal to its task. Thus the heart slowly dilates.

With progression of the dilatation the heart gradually loses the manifold advantages of normal structure, as detailed above.

As the dilatation and accompanying hypertrophy progress, the tension engendered in the endocardium rises, as demonstrated, proportional to the cube of the increasing radius. When the tension becomes sufficiently great, elastic fibers form within the endocardium. If the process is sufficiently prolonged or the tension sufficiently high, the fibroelastosis may reach major proportions.

The heart is, par excellence, the muscular blood vessel. It is comparable to the smaller muscular arteries in that, like the heart, the latter are highly important in the maintenance of systemic blood pressure. One of the characteristic responses of the

‡ The nature of the myocardial lesion in idiopathic cardiomegaly, congenital hyperplasia of muscle, is the subject of another paper, now in preparation.

smaller muscular arteries to increased pressure and stretch is the reduplication and increase of elastica in the intima (Fig. 7), representing an appearance not unlike that in the heart in endocardial fibroelastosis (Fig. 3). In short, endocardial fibroelastosis is not a unique response to increased mechanical stress, but a reaction shared by at least one other segment of the circulatory system.

Hass, in his review of elastic tissue,[29] stated:

> Thus, what investigators have learned of the phylogenesis, embryonic development, normal anatomic distribution and in vitro cultivation of elastic tissue tends to support the hypothesis that mechanical forces may exert an influence on the genesis and development of the elastica, especially if those forces are rhythmic and fluctuating.

Bunting's[30] study of pericardial adhesions and endocardial scars supports this generalization.

Plate III

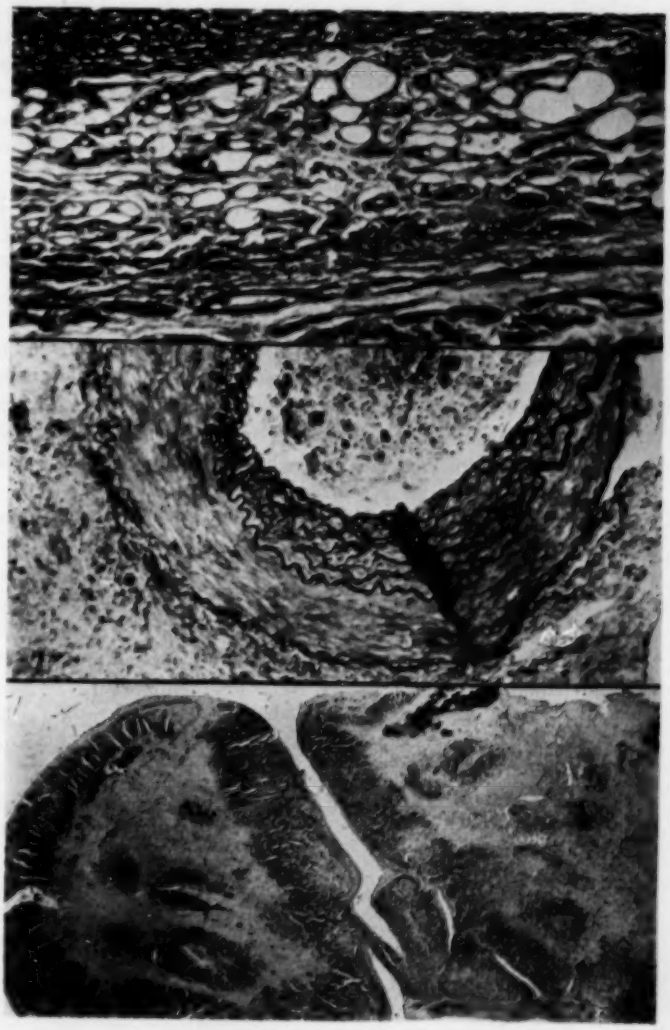


Fig. 6 (Case C52-90).—Higher power of figure 5, showing the vacuolation of myocardial fibers limited to those subjacent to the endocardium. This lesion is commonly seen in encircled trabeculae carneae (Fig. 5) and represents the severest myocardial lesion in our series.

Fig. 7.—Intimal elastosis in a renal interlobular artery characteristic of chronic hypertension.

Fig. 8 (Case N54-259; Table 6).—Left ventricle trabeculae carneae of a heart with an ectopic left coronary artery arising from a pulmonary artery. The disappearance of myocardial fibers from the central areas and fibrous tissue replacement are characteristic. There are three foci of calcification. The subendocardial muscle fibers survive.

Black-Schaffer

The proposition that fibroelastosis follows upon dilatation fits the scheme of disturbed cardiac function, since the excursion of a dilated ventricle necessary to maintain a near-normal stroke volume grows smaller as dilatation increases (Text-Fig. 2). A relatively stiff endocardium under these conditions would act less and less in the sense of a "constricting endocarditis."[31,32] In fact, it would be in the very largest of hearts with the most advanced fibroelastosis that the least deleterious effects, if any, would be manifest. If the congenital theory of origin is accepted, it is difficult to conceive how, deprived of the advantages of normal structure, these children survive as long as they do, frequently with no symptoms of cardiac failure until the week or day of death.

One other important functional consideration related to cardiac dilatation must be elucidated before further evidence in support of the above is presented. If the inner wall of the heart were a smooth surface, reduction of the ventricular volume (14 to 4 cc.) to 28.5% of its diastolic size at peak of systole would be impossible.[22] The strain of folding such an endocardium would result in its traumatization with each systole. Since the normal internal surface is highly irregular, it enables the heart to fold in upon itself in "a predetermined fashion."[25] If the internal surface, in utero, were held in a mold by the thickened endocardium and the corrugations (trabeculae carneae and papillary muscles) reduced in number and size, one would of necessity be compelled to assume that these hearts were, *de novo*, markedly dilated organs. The evidence, to be discussed later, does not support this interpretation.

Little differentiates the raw data of this material (Table 1) from those published by others, with one exception; that is the siblings 5180 and 5730.§ These were boys; the younger, #5180, was admitted to the Duke Hospital at 4 months of age with a severe upper respiratory infection. The child died soon after. At autopsy an enlarged heart, weighing 73 gm. (normal for age, 27 gm.), was found without discernible myocardial damage and showing no endocardial fibroelastosis. Some 17 months later the infant's sibling, #5730, was admitted, at age 4 months, to the same hospital because of coughing and irritability. An enlarged heart was recognized, and the infant was given digitalis. Blood pressure was measured once at 90/40 mm. Hg; EKG revealed no axis deviation but abnormal S and T waves. Repeatedly the infant was brought to the clinic with upper respiratory infections and cardiorespiratory distress. He died at 7½ months of age; the clinical diagnosis was idiopathic cardiac hypertrophy. The heart weighed 152 gm. (normal for age, 35 gm.). The left ventricle was extremely dilated and showed advanced endocardial fibroelastosis (Fig. 2). The pathologic diagnosis was "idiopathic hyperplastic cardiomegaly with left ventricle endocardial fibrosis."

If endocardial fibroelastosis were a primary defect causing cardiomegaly, how can the absence of endocardial change in one sibling and its presence in the other, both with marked idiopathic cardiomegaly, be understood? It is significant that the endocardial change occurred in the larger, more dilated, and older of the two hearts.

If our contention be true, that endocardial fibroelastosis is secondary to cardiac dilatation, an analysis of the material in Table 1 and a properly selected control group should support it.

In Table 3 are listed 22 cases with cardiomegaly but without endocardial fibroelastosis or other cardiac lesions. These were selected, as were the first 12 cases of Table 1, from the pathology protocols of the Cincinnati General Hospital and the Cincinnati Children's Hospital in the years 1949 through 1954. Only those cases were chosen in which the hearts were at least 50% heavier than the expected weight by age.[33] Of these, five were, presumably, instances of

§ Dr. W. D. Forbus, Duke University School of Medicine, permitted the use of these cases.

TABLE 3.—*Twenty-One Cases of Idiopathic Cardiomegaly Without Endocardial Fibroclastosis* *

Autopsy No.	Sex	Age	Body Wt.	Heart Wt., Gm.	Theoretical Ht. Wt. by Age	Theoretical Ht. Wt. by Body Mass	Index of Cardiomegaly Ht. Wt. / Ht. Wt. Age	Index of Cardiomegaly Ht. Wt. / Ht. Wt. Mass	Fibroelastosis	Cause of Death
N49-292	M	2½ mo.	4,578	40	23	20	1.7	2.0	–	Pneumonia and meningitis
N50-403	F	4 mo.	4,593	50	27	21	1.8	2.4	–	Enteritis
N52-97	M	10 mo.	10,433	75	39	45	1.9	1.7	–	Meningococcemia
C49-104	F	3 mo.	4,762	50	23	23	2.2	2.2	–	Encephalomalacia
C50-5	M	16 days	3,146	40	19	20.0	2.1	2.0	–	Pulmonary abscesses
C50-18	M	1 mo.	3,090	50	20	20.1	2.5	2.5	–	Cerebral edema
C50-33	M	5 mo.	7,498	60	29	29	2.0	2.0	–	Biliary cirrhosis
C51-40	M	12 mo.	8,400	66	44	35.5	1.5	1.85	–	Lobular pneumonia
C52-39	M	9 mo.	9,072	66	37	37	1.8	1.8	–	Encephalomalacia
C52-53	M	22 days	3,175	60	20	17	3.0	3.5	–	Coarctation of aorta
C53-21	M	1 mo.	3,950	75	20	20	3.75	3.75	–	Periarteritis nodosa
C53-54	M	5 mo.	5,188	61	29	23	2.1	2.7	–	Periarteritis nodosa
C53-62	M	4 days	2,890	40	20	19.4	2.0	2.0	–	Encephalomalacia
C53-70	M	2 wk.	3,250	35	20	20.5	1.7	1.7	–	Periarteritis nodosa
C54-33	F	2½ mo.	3,741	43	23	19	1.9	2.3	–	Cerebral necrobiosis
C54-36	F	13 days	3,033	43	20	20	2.1	2.1	–	Subacute glomerulonephritis
N50-556	F	20 mo.	9,541	95	56	65	1.7	1.4	–	Pneumonia
N53-105	M	17 mo.	7,158	95	50	29	1.9	3.3	–	Pneumonia with abscesses
N54-37	M	15 mo.	9,129	80	40	38	1.7	2.1	–	Bronchopneumonia
C50-11	M	20 mo.	9,600	95	56	39	1.7	2.46	–	Encephalomalacia
C50-78	M	16 mo.	6,804	95	48	29	1.9	3.2	–	Chronic pyelonephritis

* Each heart is at least 50% heavier than the expected heart weight by age.

hypertension (C53-21, C53-54, C53-70, C54-36, and C50-78). The heart in C52-53 was enlarged because of coarctation of the aorta. The cause of the cardiomegaly in the remaining 16 cases is unknown.

Since it is proposed that the primary cause of endocardial fibroelastosis is increased tension, correlated, among other things, via Laplace's law, with the degree of curvature of the endocardium, some numerical value indicating the degree of cardiac dilatation might serve to compare heart with heart within and between Tables 1 and 3.

Since few of the hearts listed were actually available and these were distorted by sectioning and fixation, useful measurements of the endocardial radii at standard loci were not possible. Even if the hearts were intact, it would seem impossible to obtain postmortem measurements that would approximate those of the in vivo diastolic left ventricle. It was believed that a rough approximation of the degree of dilatation might be given by a ratio representing the actual heart weight over its expected weight by age. Dividing this out results in an index of cardiomegaly by age. However, it is well known that children with heart disease are frequently retarded in their physical development. Since it is equally well established that cardiac mass is closely correlated with total body mass,[34-36] it was thought that a more correct index of cardiomegaly might be obtained if the expected cardiac weight by age were replaced by an expected cardiac weight by actual body mass. Thus C50-93 (Table 1), who weighed only 6293 gm. at 7 months of age (50 percentile for girls of this age is 7711 gm.[21]) possessed an index of cardiomegaly by age of 4.7; by body mass it was raised to 5.9. A χ^2 table (Table 4), constructed from Tables 1 & 3, reveals, by inspection, a significant association of endocardial fibroelastosis with the greater degree of cardiomegaly.

TABLE 4.—χ^2 *Table from Tables 1 and 3*

Index of Cardiomegaly	<2.8	>2.8	Total
With fibroelastosis	1	14	15
Without fibroelastosis	17	4	21
Total	18	18	36

$\chi^2 = 11.57$

It must again be emphasized that cardiac dilatation can be only roughly correlated with increasing cardiac weight. However, common experience indicates, in these cases, that the greater the cardiomegaly the greater the dilatation. Thus the presence of all hearts, save one, with endocardial fibroelastosis in the category of greatest cardiomegaly, and its converse, the presence of 17 of 21 hearts without endocardial fibroelastosis in the group of lesser cardiomegaly, supports the proposition advanced above.

$$\chi^2 = 11.57$$

The endocardium of nine cases of Table 1 had, histologically, been fairly well sampled. Elastic tissue stains of all blocks were examined and the degree of elastosis roughly estimated by grading + through ++++. The results are collected in Table 5. Evaluation must be cautious, since the number is small, and, even more important, the original locus of a block (not always known) is of the greatest significance.

TABLE 5.—*Nine Cases of Idiopathic Cardiomegaly with Endocardial Fibroelastosis Listed in Order of Decreasing Endocardial Fibroelastosis*

Case No.	L. Ventricle Endocardial Fibroelastosis	Heart Wt., Gm.	Index of Cardiomegaly by Body Mass	Age, Mo.
N54-122	++++	135	4.6	11
C52-90	++++	125	4.3	4
C49-79	+++-++++	110	3.5	7
N50-104	+++	104	4.8	5
N52-263	+++	90	3.0	7
N55-383	+++	100	2.8	12
C52-43	++	64	2.8	4 ½
C53-93	++	53	2.8	2 ½
886-55	+	116	5.0	3

The forces acting upon the endocardium are greatest in the left ventricle, and it would seem more than simple coincidence that it is here that fibroelastosis is most manifest. In fact, the site of earliest and most marked elastosis is near the base of the interventricular septum. This structure has the largest radius (flattest) and, by application of Laplace's law, is subject to the greatest stretch. Objective evaluation of the degree of endocardial fibroelastosis would require a large section through the entire heart in a predetermined plane. In the absence of such material, it is noticeable (Table 5) that, except for one case, S56-55, the others all fall into groups which seem to correlate directly with two simultaneously acting factors: dilatation (cardiomegaly) and duration.

Although it is proposed that endocardial fibroelastosis is a consequence of chamber dilatation, and is only indirectly related to the cardiomegaly, it is thought that the thickened endocardial membrane possesses a significant function. The additional energy needed to overcome the increasing mural tension engendered by the enlarging diastolic blood volume is ordinarily derived from a proportionally increasing muscle mass, as in normal growth of the individual. However, since this source of energy is not economical, with very large increases in diastolic blood volume it is highly improbable that hearts of the proportions of those in Table 1 could maintain systemic pressure as long as they did without some mechanism of meeting the load in addition to that ordinarily provided by the myocardium.

This source of energy, one more economical than active muscle contraction, is elasticity. This is defined as the ability to stretch and return to the original status after release of the stretching force and is exemplified by the aorta. In the normal heart elastica plays an insignificant role. However, in the abnormal heart with considerable fibroelastosis of the endocardium it probably is highly important. It is proposed that the fibroelastic endocardium serves as an auxiliary to the myocardium. It tends to slow, if not contain, the progressive dilatation. Whether the elastica is important or whether the elasticity of the collagen of the endocardium is more significant is a moot point. Of significance is the probable support it gives the myocardium. In this light the fibroelastosis may be considered not as a cause of myocardial dysfunction but, rather, as an adjunct to effective function. It supplies a counterforce to the hydrostatic pressure of the blood and the consequent stretch or tension of the heart wall. It supplies this force much more economically than could muscle, in terms of space and oxygen consumption. It is probably this internal membrane that enables the infant to maintain effective systemic blood pressure until very late in the course of his short life.

The degree of protection afforded the myocardium by an elastic endocardium may be estimated.

Assuming again, for simplicity's sake, that the left ventricle is a sphere and that the fibroelastic endocardium is a perfect elastic material which follows Hooke's Law,[37] it can be shown that

$$Tt = KR^2$$

therefore

$$Tt \propto R^2$$

Tt, as before, is the stress or tension per unit area of endocardium and is directly proportional to the square of the radius (R). K is a constant derived from Hooke's Law.

Since in our cases the wall of the heart includes both an elastic endocardium and a muscle wall, the stress will follow not the cube of the radius, as it would in the absence of an elastic endocardium, but the square of the radius. Thus if the radius is doubled, the tension per unit area, instead of reaching eight times the original (R^3), would only be four times (R^2) as great. The value R^2 represents the maximum protection, which, in fact, may be less, depending upon the endocardial elasticity and thickness.

Of inestimable value must be the greatly increased cardiac musculature. However, it could be argued reasonably that without fibroelastosis hearts of this type could not so regularly attain their huge dimensions. The cardiomegaly and the absence of any but the most minimal evidence of myocardial necrosis testify to the uninterrupted coronary blood flow despite the advanced endocardial fibroelastosis. Again, it might be suggested that, thanks to the auxiliary elastic resistance of the thickened endocardium, normal stroke volume, blood pressure, and, therefore, coronary blood flow are maintained, thus making possible the cardiomegaly.

Before conclusion of this aspect of the discussion, cognizance must be taken of a pertinent, in vivo, study of a case of endocardial fibroelastosis. Prec and Cassels [38] reported an 8-month-old infant who at birth revealed no x-ray evidence of cardiac enlargement. At age 3 months the infant, because of symptoms of failure, was again x-rayed and the picture interpreted as showing a "markedly" enlarged heart. After digitalization, an angiocardiogram was performed. It showed normal filling of the left atrium and ventricle with marked delay in outflow. The child died at 8 months of age, and the heart revealed typical cardiomegaly with left ventricular and atrial endocardial fibroelastosis. The mitral valve was mildly thickened and the chordae tendineae were shortened. Prec and Cassels concluded: "It is suggested that dilatation of the ventricular cavity results in consequence of difficulty in systolic expulsion, whatever the correct explanation of this might be, and a resulting increase in residual volume. The ultimate result is failure."

This report is of interest in that it illustrates the absence of a prime prerequisite for the diagnosis of "constrictive endocarditis," namely, difficulty in filling the chambers. One would think that the huge dilatation of these hearts would suffice to eliminate the thought of constrictive endocarditis.[39,40] Despite this, the concept not only persists but thrives.[32]

Incidence of Congenital "Idiopathic" Endocardial Fibroelastosis

There is evidence, in the literature, that "idiopathic" endocardial fibroelastosis occurs congenitally but is very infrequent. In 1946 Cosgrove and Kaump [41] reviewed 50 cases, including 6 of their own. Of the 50 infants, 23 were less than one month of age. However, of these cases, 22 were examples of either the syndrome of premature closure of the foramen ovale with a miniature left ventricle and stenosis of the aortic and mitral valves or examples of severe congenital endocardiosis with more or less endocardial sclerosis. The one remaining case without valvular lesions was reported by Jacobsthal.[42] In this report there is no mention of the endocardium. The heart was described as an example of myocarditis with myocardial calcification in a premature infant.

In the same manner, Gowing's [2] review of the literature, in 1953, revealed 29% of the cases to have died at less than one month of age. Again, perusal of the sources reveals only one of these which can be accepted as an example of uncomplicated idiopathic endocardial fibroelastosis, Case 2 described by Lewis.[43] Gowing lists the case of a term newborn reported by Simmonds [44] in 1899. Reference to the original reveals no mention of the endocardium.

It is remarkable that of the many cases thus recorded only one acceptable instance should constitute the objective evidence for a lesion widely accepted as congenital. More careful seining of the literature would undoubtedly reveal other examples of true congenital idiopathic cardiomegaly with endocardial fibroelastosis. The important point is the demonstration of its great rarity when reported cases are more critically considered.

Since the hypothesis here advanced in explanation of endocardial sclerosis is based upon mechanical considerations, cardiomegaly with endocardial fibroelastosis may

develop in utero, given the appropriate constellation of force relationships. The instances of cardiomegaly with endocardial fibroelastosis which are found in infants of less than one month of age are almost invariably complicated by severe valvular involvement. The amplification of the pressure-volume relationships in such hearts is the obvious explanation for their relatively frequent congenital appearance.

Rosahn [8] has discussed the few pairs of twins and siblings with endocardial fibroelastosis. He concluded that the lesion is possibly genetic in origin. It is suggested, on the basis of the foregoing, that the myocardium is in utero so determined that it is the source of a functional dycrasia and the endocardial fibroelastosis is a nonspecific response to chronic severe dilatation.

Congenital Anomalies with Endocardial Fibroelastosis

Two anomalies are characterized by endocardial fibroelastosis. The first is origin of the left coronary artery from the pulmonary artery or one of its branches [45-53]; the second is the complex of atretic aortic orifice and/or stenotic aortic leaflets and mitral stenosis, a miniature left ventricle, and hypoplastic proximal aorta.

Anomalous Origin of the Left Coronary Artery

The degree of cardiomegaly associated with an anomalous left coronary artery (Table 6) is comparable to that of idiopathic cardiomegaly (Table 1). In general the left ventricle is markedly enlarged, its wall thickened, and the chamber dilated. The endocardium is gray to white and thickened. The dilatation is accentuated by flattening of the trabeculae carneae and the papillary muscles. The inner third of the myocardium is fibrotic; this is most apparent at the apex. The endocardium of the left atrium reveals, in some cases, sclerosis similar to that in the left ventricle. The valves are usually not involved in the process and are generally normal. (N54-259 does reveal involvement of the mitral valve by endocardial sclerosis with production of a mild mitral insufficiency.) The right ventricle and atrium are usually not remarkable.

Microscopic examination reveals in all cases a relatively uniform appearance, namely, fibrous replacement of the muscle fibers in the inner third of the heart (Fig. 8). This is particularly true of the papillary muscles, the trabeculae carneae, and the adjacent compact myocardium. In some areas there is calcification of the centers of the trabeculae.

The endocardium is, usually, markedly thickened, and special stains reveal the presence of elastic lamellae laid parallel to the surface, as in the hearts of Table 1. Here, too, the number and thickness of the elastic fibers diminish toward the lumen. The elastosis of the endocardium is not as uniform as in the instances of idiopathic cardiomegaly. In some areas many small capillaries or sinusoidal vessels can be seen at the junction of myocardium and endocardium (Fig. 9). These are joined to larger sinusoids, which form prominent clefts in the fibrous scar tissue and can be traced here and there through the endo-

TABLE 6.—*Six Cases of Ectopic Left Coronary Artery Arising from a Pulmonary Artery*

Case No.	Age, Mo.	Sex	Body Wt., Gm.	Heart Wt., Gm.	Theoretical Heart Wt. (Gm.) by Age	Theoretical Heart Wt. (Gm.) by Body Mass	Index of Cardiomegaly by Age	Index of Cardiomegaly by Body Mass	Fibroelastosis
N50-396	4	F	5040		27				Left ventricle
N54-259	4	F	3750	56	27	20	2.0	2.8	Left ventricle and atrium
C45-65	6	F	5727	120	31	23	4.0	5.2	----
C45-90	3	...	3062	43	23	21	1.9	2.0	----
C49-11	5	M	----	80.5	29		2.8		Left ventricle and atrium
C49-73	4½	F	4550	75	28	22	2.7	3.4	Left ventricle

cardium into the ventricular lumen. Proximally, these sinusoids connect with both coronary veins and arteries. A thin layer of more or less intact but hypertrophic muscle fibers, sometimes only two or three myocytes wide, can be discontinuously traced under the endocardium throughout the areas of myocardial fibrosis (Fig. 8).

Published concepts of etiology are uniform in ascribing a primary role to the hypoxemia and the low pressure of the blood coursing through the left coronary artery. Myocardial degeneration and necrosis ensues, with healing by fibrosis, just as with infarction in the adult. The endocardial fibroelastosis has heretofore been thought to result from the abnormal blood supply, a response to chronic hypoxemia.[2,48] The thickened endocardium, presumably a result of hypoxemia, is now, in turn, made

Plate IV

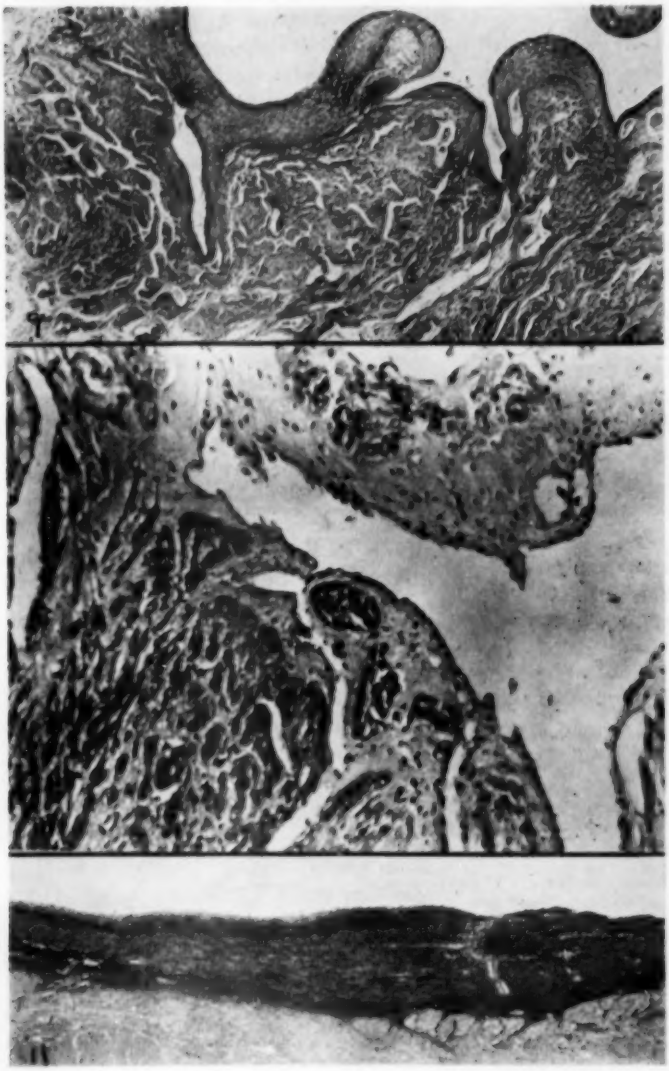


Fig. 9 (Case N49-11; Table 6).—Congerie of collateral sinusoids within the areas of myocardial fibrosis characteristic of hearts with ectopic left coronary artery.

Fig. 10 (Case C54-259; Table 6).—Penetration of endocardium by the collateral sinusoids is more readily appreciated in this higher power, although it is likewise apparent in Figure 9.

Fig. 11 (Case C53-113; Table 7).—A view of the flattened wall of a miniature, although hypertrophied, left ventricle from a heart with premature closure of the foramen ovale. The marked fibroelastosis of its endocardium is apparent.

responsible for further myocardial damage by choking off of the arterioluminal vessels and the Thebesian veins, thus adding insult to injury. This concept is the more remarkable in view of the obvious fact that the endocardium is bathed by the normally oxygenated blood in the left ventricle. That this proximity to arterial blood is of more than geographic importance is attested by the previously observed surviving layers of subendocardial muscle fibers [1,45] when all other fibers have disappeared. It is apparent that if oxygen is available from the chamber lumen for this muscle it must likewise be available to the endocardium itself.

In addition, there is the frequently observed presence of "embryonal sinusoids" forming a network of wide and narrow channels (Fig. 10) connecting the left ventricle with the coronary arteries. The concept of a "choking off" of vessels by the thickened endocardium is categorically challenged by these obviously extant and penetrating collaterals.

The term "embryonal channels" was used by Bellet and Gouley,[54] who recognized the meaning of this peculiar sinusoidal circulation because of a description by Grant, in 1926,[55] of an unusual anomaly of the coronary vessels in a malformed heart. In this same year Grant and Regnier had published a learned paper [56] on the comparative anatomy of the cardiac coronary vessels. He interpreted these sinusoidal vessels as a persistence and growth of the endothelium-clad intertrabecular spaces of the compact myocardium, which in ordinary fetal development are compressed and reduced to capillaries. In the early embryo and in adult cyclostomata and amphibians they function as an important component of myocardial circulation, especially in the cyclostomata, which have no coronary arteries. The explanation for the appearance of dilated channels in the scarred hearts of infants and adults with an anomalous origin of the left coronary artery from the pulmonary artery may be as follows: During systole the left ventricular pressure is higher than that in the coronary arteries; however, communication is shut off by the myocardial compression of the vessels. In diastole the pressure in the coronary arteries is higher than that in the ventricle, making flow into, but not out of, the ventricle possible [57]; however, since the anomalous left coronary artery carries a lower pressure than that in the ventricle during diastole, flow from the ventricle into the myocardial vessels is not only possible but mandatory. It is because of this that the channels of the normally present myocardial circulation opening into the ventricle [58] expand when the normal pressure gradient is reversed and, in addition, the extravascular support is decreased by degeneration and disappearance of the surrounding musculature. They are therefore not persisting embryonal sinusoids but analogous structures.

A literal interpretation by Proescher and Baumann [47] of the term "embryonal sinusoid" led them to suggest that the myocardial changes in their 13-month-old child had begun in utero. This curiously naïve concept of a congenital lesion due to an anomalous left coronary artery has been recently reaffirmed.[59] Since no one has described an instance of endocardial fibroelastosis associated with an anomalous coronary artery in a stillborn, or, for that matter, even cardiomegaly, and since the coronary arteries, anomalous or not, receive the same oxygenated placental blood, there is no reason to consider these views seriously.

It is unfortunate that the literature fails to convey the knowledge that in these hearts widespread progressive, acute myocardial necrosis is absent. In all six cases of this report there was nowhere an unequivocal area of fresh necrosis; the appearance was that of old healed myocardial infarction. The absence of any widespread progressive necrosis is in all probability ascribable to the congeries of arteriosinusoidal and arterioluminal collaterals.

The mechanism of cardiomegaly is generally not explained except by the use of the term "hypertrophy." In view of the facts and interpretations presented above, the following sequence of events is suggested as a result of perfusion of the left coronary artery by venous blood. Subsequent to birth the burden of maintaining systemic circulation is thrust upon the left ventricle. This entails a greater oxygen consumption by its muscles, since its diastolic volume is increased and its work load raised. However, the oxygen tension of the left coronary artery blood is low and the result is frank necrosis and/or degeneration of the inner portion of the left ventriclular myocardium. Since this decreases the strength of the ventricle, there is further dilatation. This, in turn, results in greater diastolic blood volume, with a further increment in work load. The process is again not explosive but gradual, as in the hearts of Table 1. As the radii of curvature of endocardial segments increase, the tension engendered in the endocardium likewise rises. When the heart becomes large enough, the same situation as in idiopathic cardiomegaly is established, with consequent endocardial fibroelastosis.

If, as proposed above, the endocardial fibroelastosis of idiopathic cardiomegaly is due to the mechanical stresses of severe chronic dilatation, then it may apply equally well to these anomalous hearts which, as Table 6 reveals, achieve dilatation (cardiomegaly) of the same order as the hearts in Table 1. In this respect Case C45-90, which possesses an index of cardiomegaly by weight of 2.0, showed little endocardial sclerosis, in contrast to the larger hearts.

If the process of cardiac dilatation proceeds slowly enough, and the endocardial fibroelastosis develops adequately, the myocardium will be relieved of part of its burden, thus contributing to longevity. It might be postulated that under felicitous circumstances (adequate collateral circulation) equilibrium could be achieved and the patient grow to adulthood. Such cases have been recorded.[50]

The significance of the hearts with anomalous left coronary arteries is that they present an example of endocardial fibroelastosis in which the distant etiology is evident and the pathogenesis readily understood. It emphasizes the conclusions reached in the first part of the paper, that the primary fault in idiopathic cardiomegaly is to be found in the myocardium, despite the absence of degenerative changes so clearly illustrated in the hearts with an anomalous coronary artery.

While acute infarction of myocardium may occur late in these cases, the mechanism of death as reflected by the myocardial lesion more closely approximates that of the adult with diffuse cardiac fibrosis consequent to slow occlusion of the coronary arteries with anginal attacks.

It is apropos to direct attention to the fact that these hearts are examples of advanced cardiomegaly, whose etiology is indubitably hypoxemia. The vexatious question: Can cardiomegaly be a sequel of significant hypoxemia?[60] would seem to find an unequivocally affirmative answer.

The very obvious postnatal nature of the cardiomegaly, in these cases, does serve to indicate how far some have strayed from readily demonstrated facts. It is common practice to write that hearts such as those in Tables 1 and 6 are congenitally large because of the subject's tender age at time of death. A little thought should have indicated that, in utero, the hearts with an anomalous left coronary artery must have been entirely normal and that therefore there exists no justification for the conclusion that a heart of three, or even more, times normal weight is necessarily, or even probably, congenitally enlarged because the patient is an infant. It is highly probable that this may apply to infants of one month of age as well.

Congenital or Fetal Endocardial Fibroelastosis

Congenital or fetal endocardial fibroelastosis is regularly encountered in association with a series of cardiac anomalies

TABLE 7.—*Miniature Ventricles with Endocardial Fibroelastosis*

Case No.	Age, Days	Sex	Body Wt., Gm.	Heart Wt., Gm.	Aortic Valve	Mitral Valve	Aorta	Ductus Arteriosus	Foramen Ovale	Endocard. Lt. Vent.	Fibro-elastosis Rt. Vent.	Interventricular Septum
N52-227	1	M	2000		Stenosis	Stenosis	Hypoplastic	Patent	Normal, patent	+	Hypertrophy	
C51-13	90	M		51	Atresia	Stenosis	Hypoplastic	Patent	Small, patent	+	Hypertrophy	
C51-17	2	M	4150	25	Atresia	Stenosis	Hypoplastic	Patent	Closed	+	Hypertrophy	
C52-98	8	F			Aplasia	Stenosis	Truncus communis		Patent	+	Hypertrophy	
C53-113	3	F			Stenosis	Stenosis	Hypoplastic	Patent	Closed	+	Hypertrophy	
C54-91	21	M	1580		Atresia	Stenosis	Hypoplastic	Patent	Normal, patent*	+	Hypertrophy	
C53-37†	6	M			Stenosis	Stenosis	Hypoplastic	Patent	Patent	−	Hypertrophy	Intervent. defect
					Pulmonary valve	Tricuspid valve						
C54-3	2	M			Atresia	Stenosis	Normal	Patent	Patent	Hypertrophy	+	
C53-59	6	M	2325		Atresia	Stenosis	Normal	Patent	Patent	Hypertrophy	+	

* Foramen ovale completely covered by valve but patency demonstrated.
† No endocardial fibroelastosis.

TABLE 8.—*Measurements of Myocardial Thickness of Walls of Miniature and Greatly Enlarged Functioning Ventricles with Estimates of Miniature Ventricle Volume*

Case No.	Ventricle Thickness, Mm.		Lt. Vent. Vol., Cc.	Endocardial Fibroelastosis, Left Atrium*	
	Left	Right			
C51-13	13		1-1.5	Dilated	+
C51-17	10	5	0.5	Small	0
C54-91	7	8		Small	0
C53-34	7-12	5-8			
C53-37	3- 4	3-5			
C53-59†	3	3.5			

* 0 = No endocardial fibroelastosis; +, endocardial fibroelastosis.
† Miniature right ventricle.

related to premature closure or reduction in size of the foramen ovale.[61] With diminution or cessation of the normal right-to-left shunt the only or principal source of blood for the left heart becomes the pulmonary veins. This source during fetal, especially early fetal, life is small, most of the blood being shunted away from the unexpanded lungs via the ductus arteriosus to the aorta. As a consequence, the left atrium is usually small, the mitral valve orifice is stenotic, and the valves may or may not be thickened. The left ventricle is small; repeatedly descriptions give volumetric estimates from 0.5 to 1.0 cc. (Table 8). The aortic orifice is markedly narrowed or atretic. The valves may again, as those of the mitral leaflets, be thickened and further decrease the already narrow orifice. The aorta proximal to the ductus arteriosus is usually narrowed.[62-70]

The six cases of this anomalous development of the heart listed in Table 7 illustrate all of these changes. In all cases the endocardium of the left ventricle was thickened, white, and demonstrably the seat of advanced fibroelastosis. Nowhere were degenerative changes or necrosis of the myocardium seen.

It is of passing interest to point out that some of the standard reference books[71,72] do not mention the endocardial lesion in this complex.

Since the overwhelming majority of these infants die within days of their birth, the fibroelastosis has been considered as either another anomaly or the consequence of stagnation anoxia.[3] As previously stated, no consistent attempt is contemporaneously[2,41] made to differentiate the congenital fibroelastosis of the tiny left ventricle from that of the infantile enlarged left ventricle, and the same untenable hypotheses are applied to both despite observed disparities.[3]

The diminutive left ventricle is only an appendage upon the large right ventricle and, indeed, is sometimes overlooked.[70] Nonetheless, it has been pointed out that its musculature may equal in thickness, or even surpass, that of the enlarged right ventricle.[54,63,64,66] Histologic study of the musculature in all cases reveals the fibers and their nuclei to be obviously hypertrophic, at least equal to those of the markedly hypertrophic right ventricle.

It would appear, after study of Table 7 and contemplation of what has been summarized above, that neither an anomalous development of the endocardium nor "stagnation anoxia" can be the explanation of the fibroelastosis. This emerges from consideration of Case C53-37, whose heart is identical, in all but two respects, with the six preceding hearts. There is no endocardial fibroelastosis of the small left ventricle, but there is an interventricular septal defect. The absence of the endocardial lesion in the presence of an interventricular communication has been stressed by Johnson.[3] Horley[69] writes, on this subject:

> In fact, no case of aortic atresia has been found in which fibroelastosis has occurred accompanied by an interventricular defect, and, conversely, no case of aortic atresia with an intact interventricular septum was without fibroelastosis.

It is argued by the protagonists of stagnation anoxia that the absence of fibroelastosis in such cases is due to the entrance, in utero, of right ventricular blood into the small left ventricle, thus preventing stagnation. While this may seem reasonable, it would ignore, for example, the absence of endocardial fibroelastosis from the left atria of Cases C51-17 and C53-113, in both of which there was complete antenatal closure of the foramen ovale. It is apparent that stagnation anoxia, if such exists, must have been equally present in both left ventricles and left atria. Case C54-2 and C53-59 (Table 7) show that under similar circumstances, with pulmonary valve atresia and marked tricuspid valve stenosis, the right ventricle is also extremely small and its endocardium the site of fibroelastosis. Since the coronary arteries are both supplied, retrograde, by right ventricular blood and there is no morphologic evidence of myocardial hypoxemia, some explanation other than anoxemia, stagnant or otherwise, must be forthcoming.

A reasonable explanation, consistent with the available facts, and ignoring "anoxemia" can be formulated. In the presence of aortic atresia or severe stenosis associated with severe mitral stenosis, the small left ventricle with each contraction could at best rid itself of an insignificant amount of its diastolic blood volume. If insufficiency of the mitral valve were absent and the aortic orifice completely closed (atresia), then each cycle would bring an isometric contraction of the left ventricle without reduction in size of the chamber. This places an enormous strain upon the left ventricular musculature. This is clear, since in the normal cardiac cycle the energy output of the ventricle decreases steadily from the time diastolic pressure is equaled until the peak of systole. In the hearts at issue this ebb of tension cannot occur; therefore marked hypertrophy of the small left ventricular muscle occurs, with even greater tensions exerted upon the endocardium as the muscle mass increases. The endocardial surface is smooth and the ventricle on cross section is flattened by the large right ventricle; thus the radius of curvature of much of the endocardium may be considerably larger than the small volume of the ventricle may suggest (Fig. 11). The amount of muscle acting against the small ventricular volume is enormous (Table 8). By the application of

$$T = \frac{P}{\frac{1}{r} + \frac{1}{r'}},$$

where P is very great, and r and r' likewise relatively large, the value for T may approach, if not equal, that in hearts with cardiomegalic endocardial fibroelastosis.

This mechanical concept of pathogenesis, as in the case of idiopathic cardiomegaly, is also found in the German literature. Loeser [62] believed that since the blood entering the small left ventricle could not escape, the ventricle was subjected to abnormally high pressures. This resulted in endocardial hyperplasia. Pototschnig [73] disagreed with Loeser, suggesting that the fibroelastosis was a result of faulty development and that it was the cause of the valve-leaflet stenosis. This, in turn, inhibited ventricle development. Pototschnig further hazarded the guess that the endocardial lesion was caused by the very rich blood supply visible in both myocardium and endocardium of the left ventricle.

It is of historical interest that while Loeser,[62] in 1915, established to his satisfaction that an inflammatory process played no part in the production of the valvular stenosis associated with endocardial fibroelastosis, it was not until Gross'[16] paper, in 1941, that an inflammatory or infectious etiology was finally discarded in the English language literature. Johnson,[3] the chief protagonist of the "stagnation anoxia" thesis, was influenced by his observations and the literature and wrote:

> However, in these cases, dilatation and forceful contraction of the myocardium against a relatively fixed volume of blood must also be considered as probably contributing to the production of the endocardial fibroelastosis.

Adult Endocardial Fibroelastosis

Reference [7,13-15,32] has been made, here and there in the preceding pages, to adult endocardial fibroelastosis. Since this form of endocardial sclerosis is of collateral interest to this study, comment is restricted.

While, as previously stated, most authors are agreed that infantile and adult endocardial fibroelastosis are probably etiologically different, there exists a very obvious tendency to explain the adult form as arising from the infantile or childhood lesions. Anamnestically, many of the adult patients were well until a specified time,[14,32,74-76] when they fell ill. Recovery was not complete, the patients developing chronic progressive heart failure with increasingly evident cardiomegaly. In some the early manifestations of heart disease were more insidious; nonetheless in these, as in the above, there was no historical link to early or late childhood. The untenability of the assumption that occult congenital or infantile heart disease existed is, under these circumstances, manifest. It is this hiatus in time and logic which has tempered most opinions regarding the unity of infantile and adult endocardial fibroelastosis. Despite this, the suggestion of a relationship is inevitably advanced, and not necessarily discredited.

The descriptions of the adult hearts usually emphasize myocardial fibrosis, focal distribution of endocardial sclerosis and fibroelastosis, and, less commonly, endocardial thrombosis. These characteristics differ markedly from our examples of idiopathic infantile endocardial fibroelastosis. However, these hearts are quite similar to the infantile hearts with an anomalous origin of the left coronary artery. The changes justify the hypothesis of a primary myocardial injury. Whether this injury arises as a result of a dietary deficiency [74] or of an unrecognized myocarditis [15] is unknown. At any rate, the adult cardiomegaly and endocardial fibroelastosis could satisfactorily be explained in the same manner as was the cardiomegaly and endocardial fibroelastosis of the infantile hearts with an anomalous left coronary artery.

Since the structure and function of the internal myocardial musculature are identical in the adult and the infant, all that has been written above in respect to the improbability of a primary endocardial fibroelastosis holds true for the adult, at least with one exception, which will now be discussed. The reasons already advanced against the concept of "constrictive endocarditis" in the presence of a chronically dilated chamber are equally applicable to the adult heart. However, "constrictive endocarditis" does exist in the adult. An example is the case reported by McKusick and Cochran.[39] It illustrates severe endocardial fibroelastosis of both ventricles. Significantly, both "were normal in size," and the interventricular septum was not involved. A loose layer of granulation tissue was interposed between myocardium and thickened endocardium. The presence of a subendocardial granulation tissue, the equal involvement of both ventricles, the lack of involvement of the interventricular septum by the endocardial sclerosis, and the absence of ventricular dilatation set this heart apart from the great majority reported in the literature. Obviously, mechanical factors set into motion by weakening of myocardium played no role in its pathogenesis. Löffler's [75] two cases are in many respects similar. Löffler suggested that they represented the end-results of an allergic endocarditis.

It would appear that there are multiple etiologies for adult endocardial fibroelastosis, and, whereas many of the cases may share a common pathogenesis with the infantile hearts, others do not. It is highly probable that infantile and most instances of adult endocardial fibroelastosis share no common etiology other than a weakened myocardium.

The discussion of endocardial fibroelastosis may be appropriately closed by reference to a paper by Paul and Robbins.[77] It illustrates the confusion arising from the

belief that endocardial fibroelastosis is the result of severe chronic hypoxemia. The therapy suggested is dusting (pouderage) of the pericardial cavity with magnesium silicate. While it is possible that pouderage may benefit an infant with idiopathic cardiomegaly as much as one with an anomalous left coronary artery, there is no convincing evidence in support of this view.

Summary and Conclusions

The current interpretations of endocardial fibroelastosis as a genetically determined congenital lesion and/or a consequence of hypoxemia are examined in the light of the pathologic physiology of cardiac structure and by application of Laplace's law of hydrostatics.

The material studied consists of 16 examples of idiopathic cardiomegaly with endocardial fibroelastosis, 6 cases of anomalous left coronary artery arising from the pulmonary artery, 6 instances of miniature left ventricle resulting presumably from premature closure or stenosis of the foramen ovale, and 2 cases of miniature right ventricle.

Idiopathic Cardiomegaly with Endocardial Fibroelastosis.—Woods, in 1898, demonstrated that the mechanical advantages inherent in the mammalian heart arise, in good measure, from its internal structure, which makes possible a maximal stroke volume associated with a minimal residual blood volume. Great dilatation of the heart, from any cause, results in flattening of the trabeculae carneae and papillary muscles. This alteration of normal internal structure is followed by an increase of the residual blood volume and decreased efficiency of the trabecular muscles.

The magnitude of the burden imposed upon a heart by increasing diastolic blood volume may be summarized in the following statements: With maintenance of systemic blood pressure the force exerted by the myocardium must vary directly as the cube of the radius of the chamber (the chamber being considered a sphere). Since it has been repeatedly demonstrated that "mechanical forces may exert an influence on the genesis and development of the elastica, especially if those forces are rhythmic and fluctuating,"[29] it would seem probable that the great tensions which are cyclically accentuated in the endocardium of the dilated heart would be good reason for the genesis of endocardial fibroelastosis.

Since the hypothesis here advanced is based upon mechanical considerations, cardiomegaly with endocardial fibroelastosis may develop in utero, given the appropriate constellation of force relationships. It is significant, however, that unequivocal examples of congenital idiopathic cardiomegaly with endocardial fibroelastosis are extremely rare.

Anoxemia cannot be a significant factor except terminally, since the almost complete and uniform absence of recognizable ischemic myocardial injury in these huge hearts excludes it as an etiologic factor.

The thickened endocardial membrane in the presence of a characteristically dilated left ventricle cannot possibly function, as is commonly believed, in the sense of a constrictive endocarditis. This is true, since the greater the dilatation of the ventricle the less must be the interference provided by the endocardium, for as the diastolic blood volume increases the smaller is the ventricular excursion necessary to expel a normal stroke volume. Furthermore, by simple definition a dilated chamber cannot possibly offer increased resistance to diastolic filling, as would necessarily be the case if the term constrictive endocarditis is to have any meaning. In fact, it is suggested that the fibroelastic endocardium, rather than hindering myocardial function, partly protects it from the stresses of increased diastolic blood volume.

Anomalous Origin of Left Coronary Artery from Pulmonary Artery.—In these hearts one may clearly view the factor initiating the endocardial fibroelastosis, namely, myocardial necrosis and degeneration. The pathogenesis is identical with that dis-

cussed in idiopathic cardiomegaly, since with the postnatal shift of the burden of systemic pressure the inner portion of the left ventricular myocardium dies because of a lack of oxygen. As a consequence of decreased muscle power, dilatation ensues. When this becomes sufficiently great, the infantile endocardium responds with fibroelastosis. As in the preceding category, here, too, the thickened endocardium functions as an auxiliary to the remaining myocardium. The persistence of subendocardial muscle fibers, and the characteristic presence of obvious and numerous arterioluminal collaterals in the absence of acute myocardial necrosis indicate that the thickened endocardium cannot reasonably be made responsible for aggravating myocardial hypoxemia.

Miniature Ventricle with Endocardial Fibroelastosis.—The most frequent congenital form of endocardial fibroelastosis is found in the diminutive left ventricle associated with stenotic or atretic aortic valve and stenotic mitral valve, presumably the result of premature closure or stenosis of the foramen ovale.

The absence of myocardial necrosis in these hearts and the fact that the lesion develops characteristically in utero, where a relatively hypoxemic environment is normal, would seem created to discourage those who subscribe to the "anoxic" etiology of endocardial fibroelastosis.

It is proposed that the miniature ventricle with a variously estimated capacity of 0.5 to 1.5 cc. develops endocardial fibroelastosis for the same mechanical reasons discussed in the preceding paragraphs. The small ventricle is not hypoplastic or atrophic. Measurement of its myocardial thickness reveals it to be consistently as thick as, or thicker than, the hypertrophic right ventricle. Histologically the muscle fibers are hypertrophic. In the presence of atresia or severe stenosis of the aortic valve and severe stenosis of the mitral valve, presumably without insufficiency, the pressure created in the tiny ventricle may be considerably higher than that in the right ventricle. In addition, the wall of the diminutive ventricle is flattened and possesses a considerably longer radius of curvature than its size would indicate. Thus tension in the endocardium may approach or even exceed that in the endocardium of a dilated ventricle, hence the fibroelastosis.

A mechanical explanation for endocardial fibroelastosis, whether acquired in utero or in infancy, not only is possible but appears to fit the peculiarities of cardiac structure and function.

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News and Comment

GENERAL NEWS

Harrison S. Martland Lecture.—Dr. Sidney Farber, Boston, gave the 20th annual Harrison S. Martland Lecture of the Essex County Pathological and Anatomical Society in Newark, N. J., on Dec. 5. This title was "Progress in the Treatment and Control of Cancer."

PERSONAL NEWS

Dr. John P. Wyatt Returns from Scotland Yard.—Dr. John P. Wyatt, St. Louis University of Medicine, has returned from two months' study of the coroner system at Scotland Yard, London.

Dr. Walter R. Benson Goes to the University of North Carolina.—Dr. Walter R. Benson has joined the Department of Pathology of the University of North Carolina School of Medicine as assistant professor, going there from the University of Louisville School of Medicine, Louisville, Ky.

Dr. Patrick J. Fitzgerald Presents Papers at First International Symposium of X-Ray Microscopy.—Dr. Patrick J. Fitzgerald, professor and chairman of the department of pathology, State University of New York College of Medicine, New York, presented three papers at the First International Symposium of X-Ray Microscopy and Microradiography held at the Cavendish Laboratory, Cambridge University, Cambridge, England, in August. Dr. Fitzgerald was chairman of the medical section.

Phi Delta Epsilon Lecture Given by Dr. Gustave J. Dammin.—Dr. Gustave J. Dammin, of the Peter Bent Brigham Hospital, Boston, gave the annual Phi Delta Epsilon Lecture at Yale University School of Medicine on Nov. 28, 1956. His subject was "Tissue Transplantation—Current Studies on the Survival of Skin Homografts."

Addis Memorial Medal Awarded to Dr. Jean R. Oliver.—Dr. Jean R. Oliver, of New York, has been awarded the second Addis Memorial Medal of the Los Angeles chapter of the National Nephrosis Foundation in recognition of his work on the correlation of structure and function of the kidney.

Books

BOOK REVIEWS

Lehrbuch der speziellen pathologischen Anatomie. Volume I; Part 6. By Dr. Eduard Kaufmann; edited by Dr. Martin Staemmler. Price, 42 DM. Pp. 215, with 122 illustrations. W. de Gruyter, Genthiner Strasse 13, Berlin W. 35, 1956.

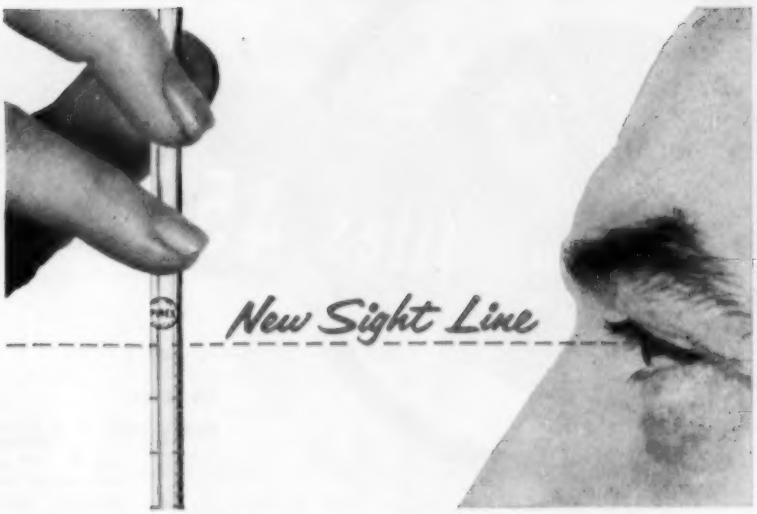
This part, the sixth of the first volume of the "new Kaufmann," deals with the pathologic anatomy of the endocrines, written by H. G. Fassbender. It is preceded by a monograph dealing with the normal anatomy and physiology of the endocrines. The various diseases are adequately discussed, and a good workable survey of the older and more recent literature is given. In some instances, the student will miss a close correlation between the morphologic appearances and the associated functional changes, since most of the latter are given in a separate part. It might have been better to discuss the histophysiology together with the anatomy immediately preceding the anatomic changes. Diseases of the islets of pancreas are included in this volume. Following old tradition, the thymus is likewise discussed here. The illustrations, a number of which are taken from negatives of the Armed Forces Institute of Pathology, are very good.

After studying all six parts of the first volume of the new "Kaufmann," I believe that this is an important addition to the texts in pathology. The editor, Martin Staemmler, so far has done an excellent job in revamping the old time-tested "Bible of Pathology," and has made a new, up-to-date work, which again will become the basic reference book of German pathologists of today. Whether it will enjoy the popularity of the old "Kaufmann" in the United States is questionable. This is due to the more difficult German and the fact that our generation is not as well versed in the German language as was the generation depending upon the old "Kaufmann." Besides, there are now excellent works on the subject written in English. Yet, for those who read the language well and are really interested in the subject, the new "Kaufmann," or, better, the "Staemmler," is a must.

Pathology Seminars. By Robert S. Haukohl, M.D., and W. A. D. Anderson, M.D. Price, $10.00. Pp. 195, with 131 illustrations. C. V. Mosby Company, 325 Washington Blvd., St. Louis 3, 1955.

This volume consists of a series of pathology seminars presented at Marquette University School of Medicine when W. A. D. Anderson was director of the Department of Pathology. Each seminar consists of a series of cases illustrative of a subject and is particularly appropriate for the person who acts as the main discussant. The discussions are informal and frequently include questions and answers.

There are tumor seminars by Lauren V. Ackerman, J. E. Ash, Arthur Purdy Stout, and Rubert A. Willis. There is, in addition, a section on the pathology of the skin by Arthur C. Allen. The material is of considerable interest and value, although some of the advantages of actual participation in the slide seminar are lost. The cases are well illustrated with microscopic material; a brief summary of each history is included, and a selected list of references is given at the end of each seminar.



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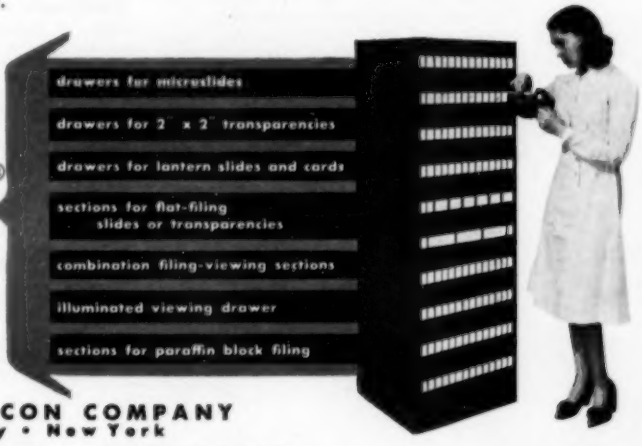

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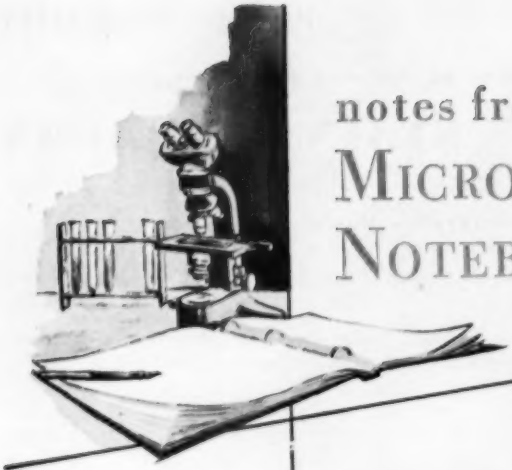

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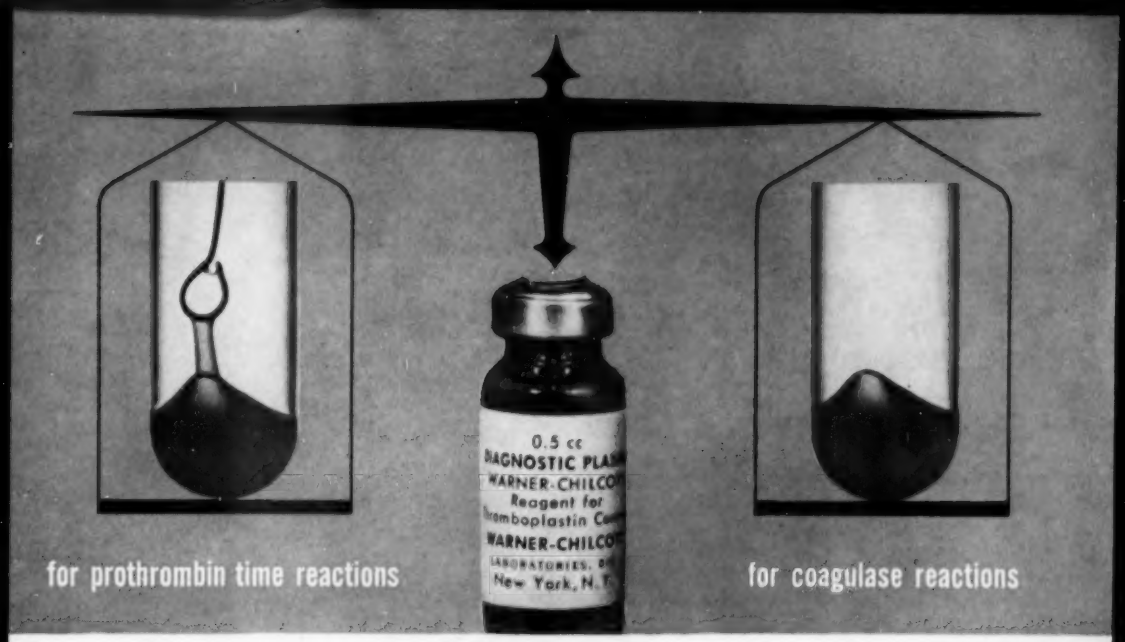

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References: 1. Tager, M.: Conference on Staphylococcal Infections, New York Academy of Sciences (Feb. 16) 1956. 2. Boyd, H.: First North American Conference of Medical Technology, Quebec (June 19) 1956.

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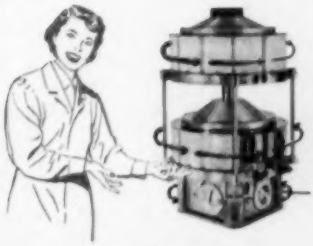

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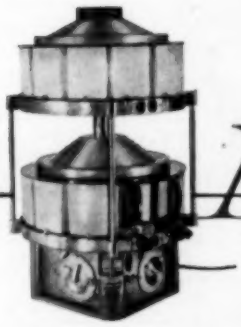

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